The Yale Journal of Biology and Medicine

The FRANCIS GILMAN BLAKE *Number*

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June, 1952

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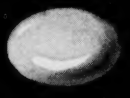

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FRANCIS GILMAN BLAKE

(Reproduced by courtesy of the Yale University Art Gallery)

FRANCIS GILMAN BLAKE
1887 — 1952

JOHN R. PAUL

Early in January 1952, only fourteen days after Dr. Francis G. Blake had relinquished his duties as Sterling Professor of Medicine at Yale and had moved from New Haven to Washington, he suffered from a severe attack of coronary disease—his second within a period of four months. Two weeks later he died in the Walter Reed Hospital. Although he may have considered his work at Yale as done, this was not taken for granted by his colleagues or his students, who, despite his departure, still looked to him as *their* chief, and still as a member of the faculty of the Yale University School of Medicine. Certainly to his closest associates in New Haven it was as if a member of their immediate family had died.

It is not enough to say that Francis Blake meant a great deal to Yale. Any graduate in medicine who received his or her degree from the Yale University School of Medicine between the years 1922 and 1952, or any House Officer who served on the Medical Service of the Hospital during that period knows for what he stood, both in the School and the Hospital. Besides his Sterling Professorship of Medicine the responsibilities which he carried on his shoulders at various times during that period included: Chairman of the Department of Internal Medicine, Physician-in-Chief and Chairman of the Medical Board of the Hospital, and Dean of the Medical School. A more detailed list appears at the end of this article. But, although this list speaks for itself, it does not tell the whole story or indicate his real influence in the School in guiding its destiny, or his influence upon clinical medicine in this country. This cannot be measured in terms of multiple positions or tenure of office.

As to his origin, he came from New England forbears and was born the son of a mining engineer in a small town in Pennsylvania. His father died when he was three years old. Most of his boyhood was spent in Massachusetts. It was no accident that as a boy he roamed the woods and developed an enthusiasm for nature, particularly for birds, or that he took a year off between college and medical school to spend the winter in a log cabin in the Maine woods, for he was a born naturalist and he published, at the age of fifteen (with his brother), his first scientific paper—on an ornithological subject. It appeared in *The Auk* in 1902.

While at Dartmouth he took all the courses in biology and zoology that he could. A dozen of his notebooks, now in the Yale Medical Library, bear witness to the extent that the science of living things took hold of him. He was never to forget biology and Dartmouth, nor did Dartmouth forget him, for he received an honorary Sc.D. from his alma mater in 1936.

At the Harvard Medical School and later at the Peter Bent Brigham Hospital in Boston he spent altogether seven years, finishing as Resident in Medicine, his opposite number in Surgery being Dr. Elliott C. Cutler. Together they had planned to become Professor of Medicine and Professor of Surgery at Harvard—a plan which did not misfire by a very wide margin.*

As to his teachers at Harvard, of all the members of the faculty whom I have heard him mention, Dr. Theobald Smith was spoken of more often than others, and always with admiration. Dr. Blake's early *medical* publications, which appeared while he was a house officer at the Brigham Hospital certainly reflect Theobald Smith's influence.

While he was at the Brigham he met and married in 1916, Dorothy P. Dewey, who was a nurse in training there. It was the happiest of marriages and no account of Francis Blake's life would be adequate without mention of his devotion to his family.

Another important phase in his education was the two and a half years (1919-21) he spent at the Hospital of the Rockefeller Institute for Medical Research in New York City, first as a Fellow in 1916 and again later as an Associate and Associate Member in 1919-1921. This was an association in which he always took pride. Nor was it a one-sided type of pride, for he subsequently served on the Institute's Board of Scientific Directors for twelve years.

At the Hospital of the Rockefeller Institute he became associated in the study of infectious disease with as brilliant and active a small group of clinical investigators in this field as had ever been gathered under one roof in this country. Among them were Rufus I. Cole, Oswald T. Avery, Homer F. Swift, and Alphonse R. Dochez; and in the adjacent Institute building were Dr. Simon Flexner, Dr. Karl Landsteiner, and a number of others. It was a period of flood tide in the clinical investigation of infectious disease and he was not alone at this stage of his life in taking advantage of it. Indeed, he was one of the most vigorous members of this group, endowed, moreover, with a special characteristic which made him stand out among the others, namely, an early appreciation of the biology of infectious disease. For it was the *nature* of infectious disease, its *natural history*, and its "philosophy," that appealed to him.

With this as a background, Francis Blake came in 1921, at the age of thirty-four to New Haven. He was one of a group selected at that time by Dean Winternitz, who were to transform the Yale Medical School within less than a decade from a second-rate institution to one worthy of the University. It was at a time when the faculty of a number of medical schools in this country, similarly situated in cities not much larger or smaller than New Haven, were being reorganized, somewhat experimentally, on a full-time basis—Rochester and Vanderbilt were among two of the other exam-

* Dr. Cutler eventually served as Moseley Professor of Surgery at Harvard.

ples. At Yale the experiment worked from the start. It was an instance of how when the right man is chosen to fill the right kind of opportunity—everybody benefits; although here as elsewhere it was not all plain sailing. There were labor pains and after-pains.

Success attended Francis Blake's efforts at Yale because he gathered at once into his department men of special calibre: Dr. W. S. Stadie and Dr. J. P. Peters to develop the metabolic field, Dr. H. M. Marvin in cardiology, and his devoted follower Dr. J. D. Trask as his own special assistant. But success was also due to his singleness of purpose, confidence in his own capabilities, and his devotion to the concept of full-time medicine. His industry and the satisfaction he derived from his work on the wards of the Hospital and in his laboratory were an inspiration to his house staff. Few realized how hard he worked. Often he told me that during the first ten years at Yale he rarely left his desk until midnight on at least four or five nights a week.

It is worthwhile perhaps to record here two of Francis Blake's concepts about full-time clinical medicine which some of his "extra-academic" colleagues and contemporaries do not seem to have appreciated. He certainly did not regard himself primarily as a "contract physician" practising "hospital medicine" for a stipend that could be measured in terms of money, although he was accused by individuals with narrower vision than his of doing just that. Not that he did not need the money. He needed it desperately, for among other obligations he had a family of three sons to raise and educate on a limited academic salary. But it was clear to him that his recompense lay in the opportunities which his position gave him, particularly that of carrying out *clinical investigation,* usually in collaboration with a junior member of his staff. Not only did it give him the opportunity to work intimately and enthusiastically with young men, but he was glad enough to raise money for *their* researches and then leave them alone to find their own way. To have participated in this form of activity was his reward. It was also participation in the progress of medicine in this country. Obviously, this ideal that he set for himself was achieved more in his younger than in his older years, for the ever-increasing burden of administrative duties and extra-clinical responsibilities eventually swallowed up the precious time he had hoped to use for clinical investigation. But I think he counted himself lucky to have had fifteen or twenty years of it.

Secondly, he had an extraordinarily clear vision as to what his functions in the Medical School were. As Professor of Medicine and as Dean of the Medical School he was beset with requests for his services or those of the Medical School to engage in various extra-academic consultations or community activities. The first question which he put to himself when he was asked to enlist his own services, or those of his Department, or those of the School, in such activities, was: "Is this a University function? If not, I shall not engage in it." For instance, he seldom engaged in out-of-town

clinical consultations, although his services were sought over and over again. To him Dr. Abraham Flexner's rugged tenets, which applied more during the 1920's perhaps than they do today, were a guide as to what a University should or should not be doing. It should not, according to Dr. Flexner give society what it wants, but what it needs. Such needs were listed as fourfold: (i) the conservation of knowledge and ideas; (ii) the interpretation of knowledge and ideas; (iii) the search for truth; and (iv) the training of students.*

Let us consider him first in the rôle of *teacher*. He certainly did not choose the easiest way here, namely, instructing by *preaching*, but he followed the hard road instead, of instructing by *example*. He was convinced that if his behavior upon the hospital wards, in the medical amphitheatre, or in the medical laboratory, was the best he was able to do, it might leave a more lasting and salutary impression on the student or house officer than any impressive pronouncements that he might make. This type of *apprentice teaching* reached full fruition with his house staff and its hierarchical system of internes, assistant residents, and resident, each one coming a step closer each year to the Chief, and each one passing a little of his hero worship down the line particularly to the third- and fourth-year students. The example that he tried to set was primarily one of integrity in thought and behavior; of carefulness rather than brilliance. When the details of a given case had been presented to him, he would often remain silent and with pursed lips proceed to examine the patient, then make his way over to the X-ray Department to see the films, then down to the laboratory to look at the blood smears or the bacteriological findings; all this with his group of "apprentices" around him, and all this to see for himself and weigh the evidence before his diagnostic decision was made. His action seemed to be in keeping with Wordsworth's lines that

> "Wisdom is ofttimes nearer when we stoop
> Than when we soar."

As a clinician, his great genius lay in the field of diagnosis. He was the ideal consultant—careful, canny, and astute, and solid as a cedar post. He could sense what was wrong with a patient over and over again where others had failed. No one ever derived more pleasure from making a correct diagnosis, and the expression which he used to assume under these circumstances was identical to that which the artist, Mr. R. P. R. Neilson, has caught in the portrait painted in June 1951, six months before his death.†

In the care of his patients he was chary with words, cautious with the use of any but specific drugs with constant warnings to his assistants about the dangers of unnecessary drug therapy which might result in "meddle-

* Flexner, A.: *Universities: American, English, German.* New York, Oxford University Press, 1930, p. 6.

† See the frontispiece of this volume.

some treatment." But he was also a past master in the use of specific therapy, having lived through and contributed materially to three eras in the treatment of infectious disease: the era of antiserum, of chemotherapy, and of antibiotic therapy. The writer will not soon forget Francis Blake's excitement in the late 1930's during the early days of the use of sulfonamides. He set the pace for the use of the sulfonamide derivatives, to which his bibliography during the late 1930's bears witness. And the writer will also not forget seeing him one afternoon in June 1941 in earnest conversation with Dr. Florey (later to become Sir Howard) during the latter's wartime visit from England to New Haven. Six months thereafter Dr. Blake was to administer one of the first doses of penicillin given in this country. Nor will the writer forget being Francis Blake's patient once during a long illness which kept him in bed at home for four weeks. Dr. Blake never missed a day in visiting his patient during that period.

As a *clinical investigator,* he led the field. Here he was in his real element, having been drawn, as any true medical biologist might, into the clinical applications of microbiology and the study of infectious disease. It was a field which was to become in a large measure transformed during his lifetime—and in this transformation he was to play no small part.

If he had one special or particular interest in medicine it was with acute respiratory disease, notably influenza and pneumonia, but his interests covered a wide range, as can be seen by a glance at his bibliography, which lists almost 140 published articles. He turned his attention sequentially to several special projects:

(i) The production of pneumonia experimentally—and ways and means of preventing it. Here he participated in an extensive series of experiments carried out in an Army laboratory in Washington with Dr. Russell L. Cecil immediately after World War I.

(ii) The virus etiology of measles, which was demonstrated in a classic series of experiments carried out on monkeys at the Rockefeller Institute in 1919-1921 in collaboration with his junior colleague, the late James D. Trask. The solidarity of the measles research team of Blake and Trask was such that Dr. Trask said that when Dr. Blake was invited in 1921 to become Professor of Medicine at Yale, there never was a question in his own mind but that he would go too, even if he had to go as a technician. Their experiments proved once and for all that measles was caused by a virus to which rhesus monkeys were susceptible. To remind Dr. Blake of this, a picture of a rhesus monkey spotted with the rash of measles hung above his laboratory desk for at least thirty years.

(iii) In the early 1920's, studies on scarlet fever were carried out by Blake and Trask on the isolation wards of the New Haven Hospital. It was the logical application to clinical medicine of Dochez's discovery that certain strains of hemolytic streptococcus produced an exotoxin and at

times a demonstrable toxemia which could be treated successfully by scarlatinal antitoxin. The work did not receive the universal recognition it deserved.

(iv) The possibility of the use of pneumothorax in the treatment of acute pneumonia.

(v) Chemotherapeutic agents and antibiotics. At least twenty-seven papers listed in his bibliography deal with this subject.

During World War II, as President of the Army Epidemiological Board, he accompanied as director, a team of investigators which went to New Guinea, while it was still technically in the combat zone, to study scrub typhus (or tsutsugamushi disease). This was an important cause of serious illness among troops in action in the South Pacific, and American scientists had very little first-hand knowledge at that time about the disease, its vectors, and animal reservoirs. The information procured by Dr. Blake's team was the basis of much subsequent work by American investigators.

This chance to visit New Guinea came at a time when he was overwhelmed with teaching and administrative responsibilities, both at home and in Washington. Actually, it was a welcome break, for a letter written from New Guinea by Dr. Blake tells of his great pleasure in being able to spend several "quiet" weeks working all day in a special ward of a field hospital. He had had to travel far in the South Pacific during wartime and at considerable risk to find this opportunity. He returned to New Haven with a wealth of new data on scrub typhus, a wonderful collection of butterflies from New Guinea, but he also had had the bad luck to acquire tertian malaria from which he suffered recurrent attacks during the subsequent two years.

As an administrator he worked with dispatch and with no fuss. Seldom did he let administrative details keep him at his desk after 10:00 a.m., for he was due on the hospital wards at that time—even when he was Dean of the Medical School.

And finally this account would be inadequate without mention of Francis Blake's long record of service to his country. This was one kind of extra-academic activity that he did not shun; perhaps it may have later become part of his philosophy of "teaching by example." Serving first as a Medical Officer in the Army in World War I he got to know the ways of the Army Medical Department and of Washington where he became very much at home. As a result he was called upon constantly by the Army and other governmental agencies for the rest of his life. During the 1930's he was tireless in his work for the National Research Council and it was at that time that it became evident to other Washington agencies that here was a man of wise counsel. From 1945 onwards the Surgeon General's Office of the Army, the National Research Council, the U.S. Public Health Service, and the Office of Scientific Research and Development in the Army turned

to him more and more. But his greatest achievement in Washington was the organization and direction of the Army Epidemiological Board of which he was the first President—an office that he held for six years. The concept of this semi-military organization which utilized but did not exploit university personnel was hatched in 1940 jointly by General (then Colonel) J. S. Simmons and Francis Blake. It proved to be a most effective and closely knit organization, based on the fact that it was not an advisory board per se, but a working group armed with contracts to carry out basic and applied research at home and in the field. The citation with which he was honored when he received for this achievement the Medal for Merit in January 1946 is not a series of overstatements, it is singularly accurate.*

How one man could work so quietly and effectively over the years, how he could possess so many different capabilities—research worker, university administrator, clinician, teacher, wise committeeman and Board member—and yet excel in all of these, is difficult to understand, but it inspired reverence akin to worship in all who knew him.

His death at the age of sixty-four came with appalling swiftness. To his friends he had not been the type of person whom one might suspect of developing coronary arterial disease, so his first heart attack and subsequent fatal attack were a shock of the first magnitude. Only after he had gone did one begin to realize the full extent of his beneficent influence. One also realized that:

"when the stream
Which overflowed the soul was passed away,
A consciousness remained that it had left
Deposited upon the silent shore
Of memory images and precious thoughts
That shall not die, and cannot be destroyed."

* "Francis Gilman Blake, A.B., M.A., M.D., Sc.D., eminent physician and scientific investigator, leader in curative and preventive medicine, wise and able administrator, devoted servant of the health of the Armed Forces, for exceptionally meritorious conduct in the performance of outstanding services as Consultant to the Secretary of War, and President of the Army Epidemiological Board. Through his organization and direction of the Army Epidemiological Board since its establishment by the Secretary of War in January 1941, Dr. Blake has guided investigations, policies, and measures which have safeguarded the health of troops. He has directed research and the applications of research which have resulted in decrease of infectious diseases at Army posts and stations in this country and overseas and which have led to development of new protective measures. He has administered the Army Epidemiological Board as a means for preparation in advance of the outbreak of the war and as an effective weapon to combat communicable diseases during the war. In addition to his constant advisory assistance and supervision of the activities of the ten Commissions under the Army Epidemiological Board he has contributed benefits by his own personal participation in field work, under campaign conditions, to the understanding and control of scrub typhus fever, one of the most serious hazards to the health of troops in the Southwest Pacific Area. In his capacity as trusted adviser on the health of the Army and as leader in investigation and control of influenza and other epidemic diseases in the Army, Dr. Blake has rendered courageous and exceptionally meritorious service to the nation."

Curriculum Vitae

1887 Born (February 22), Mansfield Valley, Pennsylvania.

1908 A.B., Dartmouth College.

1913 M.D., Harvard Medical School.

1913–16 Successively: Medical Interne, Assistant Resident, and Resident, Peter Bent Brigham Hospital, Boston, Massachusetts.

1916–17 Moseley Traveling Fellow, Harvard University.

1917–19 Assistant Professor of Medicine, University of Minnesota Medical School.

1918–19 Served on active duty (during World War I) as 1st Lieutenant, Captain, Major, Medical Reserve Corps, U.S. Army.

1919–20 Associate in Medicine, Hospital of the Rockefeller Institute for Medical Research, New York.

1920–21 Associate Member, Rockefeller Institute for Medical Research, New York.

1921–52 John Slade Ely Professor of Medicine (1921–27), Yale University School of Medicine. Sterling Professor of Medicine, 1927–1952.

1921–51 Chairman of the Department of Internal Medicine, Yale University School of Medicine.

1921–51 Physician-in-Chief, New Haven Hospital (and later, 1946-1951) the Grace-New Haven Community Hospital, University Service.

1921–51 Physician-in-Chief, New Haven Dispensary.

1921 M.A. (Honorary), Yale University.

1924–35 Member of the Board of Scientific Directors, Rockefeller Institute for Medical Research.

1925–36 Member, Division of Medical Sciences, National Research Council (Chairman of the Division for the term 1933–36).

1928–51 Chairman, Medical Board of the New Haven Hospital, and later (1946–51) the Grace-New Haven Community Hospital.

1931 President, American Society for Clinical Investigation.

1932–33 Member, Executive Council, Association of American Medical Colleges.

1934–52 Associate Fellow of Silliman College.

1935 President, American Association of Immunologists.

1936–46 Chairman, Medical Fellowship Board, National Research Council.

1936 Sc.D. (Honorary), Dartmouth College.

1938 Chairman of the Section on Medicine, American Medical Association.

1939–47 Regent, American College of Physicians.

1940–41 Acting Dean, Yale University School of Medicine.

1940–52 Member, Committee on Chemotherapeutic and other Agents, National Research Council (Committee on Chemotherapy, 1946–52).

1941–47 Dean, Yale University School of Medicine.

1941–46 Consultant to the Secretary of War and President of the Board for the Investigation and Control of Influenza and other Epidemic Diseases in the Army, War Department, Preventive Medicine Service, Office of the Surgeon General, U.S. Army; known from 1946 to 1949 as the Army Epidemiological Board.

1943 During World War II, on Expedition to New Guinea to study scrub typhus fever.

1943–47 Special Consultant to the Committee on Medical Research, National Research Council.

1943–46 Member, Subcommittee on Coordination of Malaria Studies, National Research Council.

1945–50 Chairman, Advisory Council, Life Insurance Medical Research Fund.

1945 Received Charles V. Chapin Memorial Award from the City of Providence, Rhode Island.

1945 Awarded United States of America Typhus Commission Medal.

1946 Awarded United States of America Medal for Merit, the highest civilian honor.

1946–52 Member, Army Epidemiological Board, known since 1949 as the Armed Forces Epidemiological Board.

1946 Vice-president and Chairman, Section on Medicine, American Association for the Advancement of Science.

1946–48 Member, Advisory Board on Health Services, American Red Cross.

1947 Elected to National Academy of Sciences.

1947–52 Member, Committee on Medicine, National Research Council.

1948 Vice-president, American College of Physicians.

1948 Elected Fellow, American Academy of Arts and Sciences.

1948–51 Member, National Advisory Health Council, U.S. Public Health Service.

1948–51 Chairman, Committee on Medical Sciences, Research and Development Board, National Military Establishment.

1949 Elected Member, American Philosophical Society.

1948–49 President, Association of American Physicians.

1950 Participated in giving lectures in Australia under the Australian Postgraduate Federation in Medicine.

1952 Technical Director of Research, Medical Research and Development Board, Office of the Surgeon General, Department of the U. S. Army.

1952 Died February 1 in Washington, D. C.

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THE STUDY OF SERUM PROTEINS AND LIPIDS WITH THE AID OF THE QUANTITY ULTRACENTRIFUGE

VII. Some Features of a System of Lipoproteins which Contain Phospholipid but No Free Cholesterol*†

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We propose in the present paper to describe a system of lipoproteins which contain phospholipid but no free cholesterol and little or no esterified cholesterol. We utilize data which we have accumulated during the past four years during which time we have studied serum proteins and lipids by making multiple analyses of ten samples of each ultracentrifugate of untreated serum. The samples of centrifugate from standardized positions in the column are designated by numbers beginning with number one at the top, which is the cream layer sample.[7] The detailed data have been published elsewhere,[7, 8] or are in process of publication.[3, 4, 6, 9, 10]

The accumulated evidence from many sources, as reviewed by Edsall,[2] indicates that serum lipids are in the main associated with proteins. Our data concerning the distribution of lipids and proteins in the ultracentrifugate provide both general and special support for that concept. Our finding, in low density centrifugate, of a concentration of globulin in excess of that expected from the albumin : globulin ratio of whole serum, along with high concentrations of lipids, strongly suggests that the globulin is there in increased concentration because of buoyancy provided by lipids attached in some fashion to the globulin molecule.[7] Conversely, the finding of high concentrations of phospholipid and neutral fat in high density centrifugate indicates the attachment of these low density lipids in small proportions to many high density molecules. More specific information bearing upon this subject is provided by the systematic relationship between total globulin concentration and lipids at certain levels in the upper half of the column which we interpret as indicating stoichiometric relationships between proteins and lipids.[6]

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† Some of the data in this paper were presented to the Association of American Physicians on May 6, 1952.

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It is necessary that we define some of the terms used in this paper, especially that we make clear which lipids seem in a strict sense to be a part of protein molecules. Only in the cream layer sample have we observed any systematic relationship between neutral fat and any other lipid component, while there is abundant evidence for such relationship among the other three lipid components we have measured in the top five samples of normal centrifugate. Because it is difficult to imagine that so much neutral fat is incorporated into the few protein molecules present in the cream layer sample, we are inclined to discount the stoichiometrical data for neutral fat even there and have adopted tentatively the generalization that neutral fat is attached to lipoprotein molecules by some physical bond rather than by incorporation into the molecule. We have come to use a system of terminology based on the assumption that the two forms of cholesterol and phospholipid are incorporated in different combinations in protein molecules and that these same molecules establish some physical union with neutral fat to form lipoprotein-fat complexes.

Our evidence has led us to the hypothesis that there are two major systems of lipoproteins according to their lipid composition, apart from fat, and one minor system to which we assign a small fraction of cholesterol ester which has a special distribution in the centrifugate. We imagine that the lipoproteins in all of these systems may transport fat and we have evidence that the two major ones do so. The proportion of fat per unit of lipoprotein is believed to be highly variable, and in disease states and under dietary stress sedimentation characteristics may vary because of large differences in fat load. The lipoprotein of greatest complexity of lipid composition, according to our evidence, is one which contains globulin, phospholipid, and both forms of cholesterol in stoichiometric proportions, and is found in normal centrifugates in two major concentrations, the cream layer sample and the mid-zone. In this latter zone the distribution curve along the column has the general shape of a normal frequency distribution curve, that of free cholesterol.[7] The proportion of cholesterol ester relative to free cholesterol differs in the two principal concentrations, that in the mid-zone being twice that in the top sample. The relationship between free cholesterol and phospholipid, however, does not vary significantly from one part of the column to another.[8] We refer to the protein of one major system as phospholipid-cholesterol protein (PL-C-protein), and to that of the other major system as phospholipid protein (PL-protein). We emphasize the absence of free cholesterol from the protein of the second system and for the time being assume that this system contains no ester as well. The second system is therefore conveniently referred to also as that which has phospholipid without cholesterol. There is a possibility that two or more components of this system may actually contain the small fraction of cholesterol ester which has a distinctive distribution, but, partly for convenience in terminology and until more definite evidence is available, we assign this fraction

of ester to a third and separate system of lipoproteins which we call the cholesterol ester proteins (CE-proteins). This fraction of ester occurs in two concentrations, the larger deep in the column along and beyond the high density tail of the free cholesterol distribution curve, and the smaller one high in the column, chiefly in the third sample. In these two regions there is cholesterol ester in excess of that expected by the strict proportionality with free cholesterol exhibited in other parts of the column.[8] According to our terminology, union of any of these lipoproteins with fat

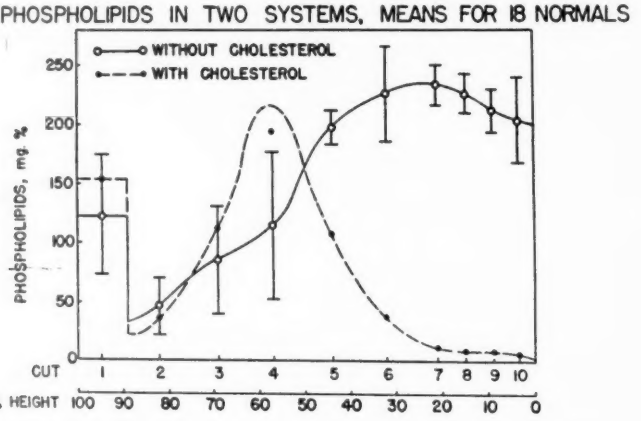

FIG. 1. Mean curves of distribution of phospholipid assigned to two systems in centrifugates of 18 healthy young men. The abscissa represents volume of centrifugate according to position in the centrifuge tube. Figures 1-10 indicate the centers of each of the ten standard samples. The centrifuge tube may be imagined as lying horizontally with the bottom toward the right and the top at the left. The vertical lines on either side of the curve for the PL-protein system indicate one standard deviation. Large values for S.D. in number 6 sample are possibly attributable to the fact that this sample was frequently collected in two parts with consequent increase in error.

gives a protein-fat complex characterized by the specific lipoprotein and by localization in the column.

The phospholipid proteins devoid of free cholesterol are the principal subject of the present paper. We shall give descriptive data for the PL-C-protein system only when comparison is especially helpful in describing the system without cholesterol. Proof for the existence of lipoproteins which contain phospholipid but not free cholesterol is provided by the fact that about thirty-five per cent of lipid phosphorus is found in the high density one-third of the column of centrifugate where insignificant quantities of free cholesterol are found (Fig. 1). At higher levels where significant concentrations of free cholesterol occur, differentiation of the two systems of phospholipid-containing lipoproteins is entirely a mathematical procedure.

We have found a linear relationship between concentration of free cholesterol and phospholipid for a group of samples from each of the upper five positions in the column of normal centrifugates.[8] In no instance does the line of regression pass through the origin, indicating phospholipid in excess of that necessary to meet the requirements set by slope of the line (Fig. 2). This excess varies widely with position in the column and seems to be related to the mean concentration of phospholipid without free cholesterol. The general features of the graph for a given level in the column of centrifugate are similar to those published by Albrink, Man, and Peters for

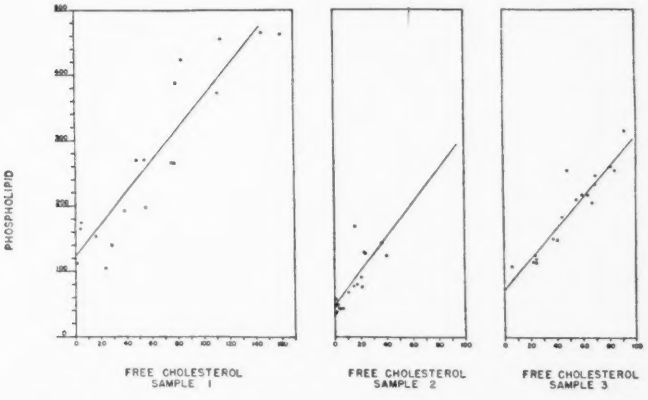

FIG. 2. Spot graphs in which values for free cholesterol are plotted against those for phospholipid (in milligrams per cent) for 18 samples from each of the three upper positions in the column, centrifugates of 18 healthy young men. (Transactions of the Tenth Conference on Liver Injury of the Josiah Macy, Jr. Foundation, New York, May 1951, Fig. 2, p. 19.)

whole serum.[1] Our graph for a group of samples from some levels, for example the third from the top, shows points with such small abscissae and large ordinates as to exclude the possibility that the line might curve and pass through the origin (Fig. 2).[8] The slope of the line differs somewhat for whole serum and for groups of samples representing different levels in the column but not significantly as evaluated by statistical methods. This demonstration of linear relationships with limited variation in slope at several levels after radical rearrangement of lipoproteins and with wide differences in intercept values is strong evidence for the existence in the upper five samples of centrifugate of phospholipid in two states, in one combined with free cholesterol in stoichiometric proportion and in the other uncombined. The concentration of phospholipid in the second state tends toward constancy at a given level in the column. The variation in concentration of phospholipid without free cholesterol in normal centrifugates at any one

level, however, is believed to be sufficiently great to prevent highly accurate estimation of the constant which expresses the stoichiometrical relationship between free cholesterol and phospholipid in the PL-C-protein. In the data used in the present paper we have assigned phospholipid to two systems, level by level, in centrifugates according to the formula for each level. After extensive application of this simplest possible interpretation of the systematic relationship between free cholesterol and phospholipid to centrifugate data from a wide variety of sources we have gained confidence in its truth, without any assumption of final accuracy for the mathematical procedure. Just as considerable reproducibility of concentration of phospholipid without free cholesterol in a group of samples from a given position in the column appears to be essential to demonstration of linear relationship between free cholesterol and phospholipid in centrifugate (the line of regression not passing through the origin), a similar constancy of concentration in whole serum might be essential for the relationship shown by Albrink, Man, and Peters.[1] Evidence that such is the case will be presented in this paper.

A constant multiplied by the concentration of free cholesterol gave concentration of phospholipid in the PL-C-protein system in a given sample of centrifugate. That assigned to the PL-protein system was obtained by subtraction from total concentration of phospholipid estimated in that sample. Phospholipid in each system in whole serum was calculated from data for the centrifugate.

The two curves in Figure 1 show mean distribution of phospholipid in 18 centrifugates of healthy young men assigned to the two major systems of lipoproteins. The size of the area beneath one of these curves is proportional to the total concentration of phospholipids in that system in whole serum. The shape of the area describes the constituents of the system mainly according to density of lipoprotein complexes of which it is constituted. In the centrifugates of healthy young men there is considerable reproducibility of both size and shape of the areas representing both systems. As a result of stress of a large meal or of disease a system pattern may deviate significantly from the normal, either as to size or shape or in both ways. The mean concentration of phospholipid in the PL-C-proteins of whole serum of 18 healthy young men was found to be 80 mgs. per cent with a standard deviation of 21.1 and that in the PL-proteins (without free cholesterol) 150 mgs. per cent with a standard deviation of 16.4. When standard deviation is expressed as percentage of the mean, that for the PL-C-protein system is found to be more than twice that for the system without cholesterol, being 26% as compared to 11%.

The tendency toward constancy of concentration of phospholipid in whole serum assigned to the system without free cholesterol as compared to the variability in the cholesterol-containing system is even more impressive when the sera of a group of miscellaneous patients are considered. Such a

comparison is shown in Figure 3 which represents the results of analyses of centrifugates of 47 sera expressed as phospholipid assigned to the two systems. This group of patients includes a disproportionate number, 13, with diseases of the liver and biliary tract, two or three each with diabetes, nephrosis, and thyroid disease, and the remainder with miscellaneous diagnoses without particular reference to known disturbances in serum lipids. In general, the graph shows a strong tendency toward wide scatter and increased concentration of phospholipid in the PL-C-protein system and a

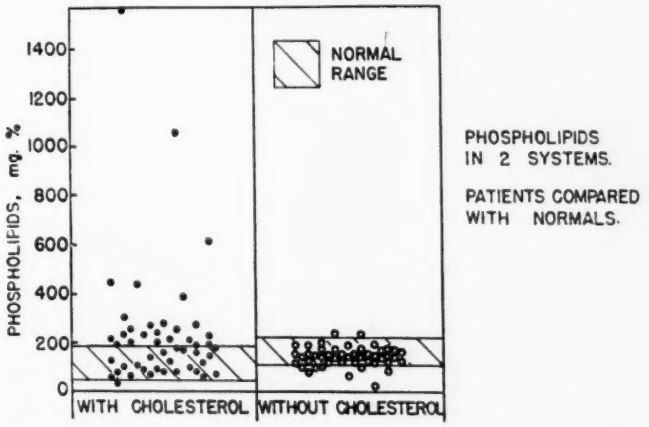

FIG. 3. Comparison of phospholipid concentration assigned to the PL-C-protein system (with cholesterol) with that assigned to the PL system (without cholesterol) as determined in centrifugates of 47 patients with various diseases.

tendency toward small scatter and diminished concentration of phospholipid in the system without free cholesterol. The normal ranges indicated by the cross-hatched areas were established by the maximum and minimum values obtained from study of 30 apparently healthy individuals, including a number of sera with concentration of neutral fat which exceeded our standards for normal and therefore were not included in the strictly normal series of 18 referred to above. In summary, our comparison of the two serum phospholipid compartments as to size indicates that the one without free cholesterol is the larger and more constant in the post-absorptive state in health, and that in disease it also varies little from normal size. When it does, it is predominantly toward diminution in the diseases we have studied. On the other hand, phospholipid in the system with free cholesterol varies more widely both in health and in disease, principally toward increase, containing occasionally in disease more than ten times as much phospholipid as the system without cholesterol.

As shown graphically in Figure 1, there is considerable reproducibility of distribution in the centrifugate of the different components of the system of lipoprotein which contains phospholipid without free cholesterol. As we have reported elsewhere,[7] the reproducibility of specific gravity for our standard ten samples of centrifugate of the serum of healthy young men is the greatest of any measurement we have made. There is abundant evidence that the lipoprotein-containing particles tend to localize in the column from top to bottom according to increasing density of complexes. We have

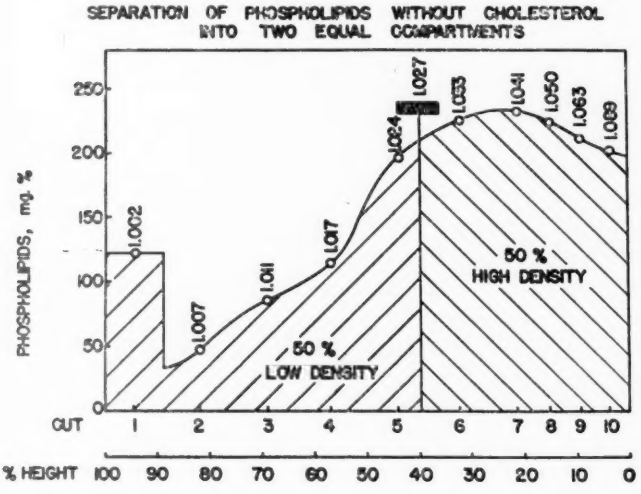

FIG. 4. Graph showing average position of a hypothetical plane which separates phospholipid in the PL-protein system into two equal compartments, 18 normal centrifugates. Localization according to specific gravity of centrifugate is 1.027, S.D., 0.003. Figures above the curve at each sample level represent the mean specific gravity.

measured the density of lipoprotein complexes by resuspending samples of centrifugate of known specific gravity and composition in a series of sucrose solutions of varying density and determining the distribution of the different lipid components after ultracentrifugation of the mixture. Those found in the top one-quarter of the sucrose column were considered as less dense than the medium from which they rose and those in the bottom one-quarter, conversely, as denser than the medium from which they were sedimented. The lipid complexes of samples from the upper one-half of the column were found by this test to have a density closely and systematically related to the specific gravity of samples of centrifugate in which they were found after the original centrifugation of untreated whole serum. Similar experiments with samples from the bottom one-half of the column were less satis-

factory and more difficult to interpret, perhaps because of the high concentrations of sucrose which were of necessity used.

While recognizing the possible importance of particle size and shape upon the distribution of lipids in the column of centrifugate we believe that particle density is the most important characteristic in determining localization in the column of lipoproteins. According to this concept we believe that the distribution of the components of the lipoprotein system containing phospholipid but no free cholesterol gives a description of the components in terms of sedimentation characteristics in which particle density is most important. The curve of distribution of phospholipid in this system (Figure

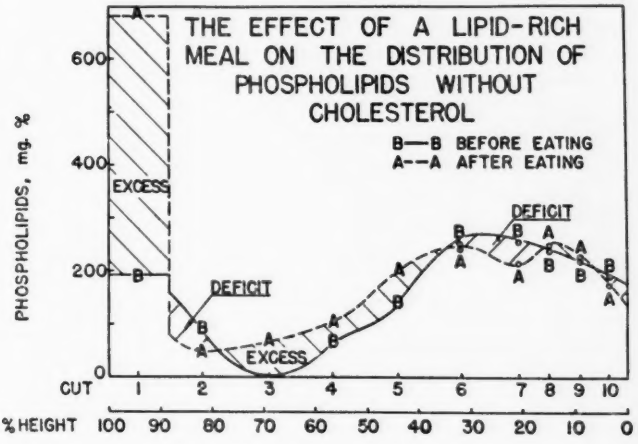

FIG. 5. Curves of distribution of phospholipid in the PL-protein system in two centrifugates in the same individual. B, post-absorptive sample taken before lipid-rich breakfast, A, 3 hours after eating.

1) shows one standard deviation on either side of the mean curve indicative of the degree of reproducibility of the curve of distribution at each level. The results of another test of reproducibility are shown in Figure 4. For each of 18 centrifugates in our strictly normal series we determined the location in terms of specific gravity of a plane across the centrifuge tube which would separate phospholipid without cholesterol into two equal compartments. The partition shown in Figure 4 is the mean value obtained, which is at a specific gravity of 1.027 with a standard deviation of 0.003. The mean phospholipid in centrifugate of specific gravity 1.027 and greater was 48.7% of the system total with standard deviation of 6.6. As tested in this fashion, the quantity ultracentrifuge tends to distribute the components of this system with remarkable accuracy so that one-half of the phospholipid is found below a hypothetical plane at a specific gravity level of 1.027 and

the other half above this plane. The high degree of reproducibility in the concentration of the different components of this system of lipoproteins is, we believe, strong evidence that these different components make up a system which has physiological features in common and which possesses also the structural feature of containing phospholipid without free cholesterol. Furthermore, the tendency toward constancy of concentration of the system as a whole and of the different components further suggests control by regulatory mechanisms of impressive efficiency.

We present two examples of deviation from the normal pattern of distribution of the components of our system, one resulting from a physiologi-

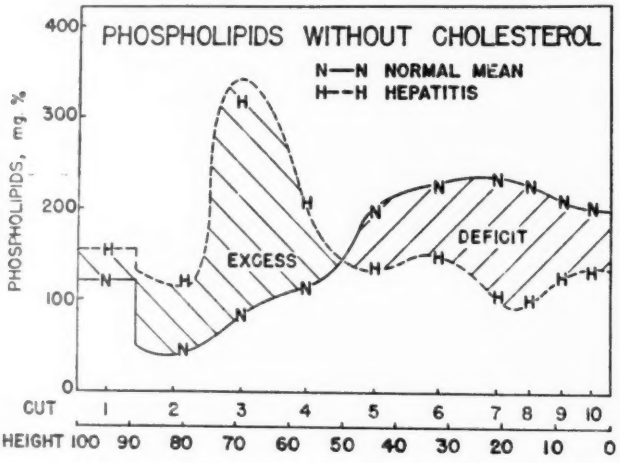

FIG. 6. The curves of distribution of phospholipid assigned to PL-protein system, H curve representing one typical hepatitis centrifugate, N curve representing mean normal distribution for 18 healthy young men.

cal stress, that of taking a lipid-rich meal, and the other a pathological stress, namely, the disease, acute hepatitis, presumably of virus origin. In Figure 5 are shown curves of distribution of phospholipid without cholesterol which are typical of our observation on alimentary lipemia.[5] The two centrifugates are from the same subject, one from the bleeding before breakfast, the other three hours after having eaten at a time when the serum was milky in appearance. The breakfast included bacon, eggs, butter, cream, and chocolate. The principal change was found in the top sample where the well-known increase in fat was localized. Because the concentration of phospholipid and fat was increased out of proportion to that shown by the two forms of cholesterol which maintained their usual proportionality for this sample, we conclude that there was an increase in the system which is

devoid of cholesterol and that a substantial portion of the fat in that part of the column is carried by this system.

In Figure 6 the distribution of phospholipid not associated with free cholesterol in one centrifugate of serum from a patient with acute virus hepatitis is compared to the mean normal distribution.[10] In this instance the total concentration of phospholipid in the system under discussion falls within the normal range, but obviously the distribution is abnormal. There is an increase which is extreme in the third sample and quite marked in adjacent samples and a diminution of varying degree in all samples in the high density one-half of the column, including the fifth to the tenth samples, the excess in the low density half of the column being approximately equal to the deficit in the high density portion. The increase in the top sample in this centrifugate is not typical of hepatitis centrifugates, which commonly show diminution.

In our search for a mechanism to explain the distortions of patterns such as these we have just described, we are impressed by the increase in neutral fat in that portion of the column where the increase in phospholipid concentration is found and with the possibility that increased proportion of fat in lipoprotein-fat-complexes provides sufficient buoyancy to the particles to bring about their localization at a higher level in the column than would occur with normal content of fat. In normal contrifugate only 20% of neutral fat was found in the second to fifth samples, inclusive, but in ten hepatitis centrifugates a mean of more than 60% of serum fat was found in the comparable region. It is in this portion of the column, especially in the third sample of hepatitis centrifugates, that a tremendous increase in phospholipid assigned to both systems was found.[10]

The stresses produced by heavy eating of lipids and those found in acute hepatitis result in an increase in proportion of low density lipoprotein complexes, both systems being affected. The localization in the column of the excess is characteristically different for the two conditions although excess fat makes up a large part of the increase in both. This suggests that the attachment of fat to lipoproteins is affected by controlled and systematic processes which may be highly specific as to the proteins participating, the quantity of fat attached, and the mode of attachment.

We have pointed out elsewhere[1] that the concentration of phospholipid in the bottom one-third of normal centrifugate has the highest degree of reproducibility of any lipid component. Practically all of the phospholipid in that portion of centrifugate is without free cholesterol. This constancy is well maintained under the physiological stress upon the lipid transport system consequent to ingestion of a lipid-rich meal, but not in acute liver disease. A deficit of high density PL-proteins along with an excess of those of low density is the rule in hepatitis centrifugates.[10] Shrinkage of the system as a whole was observed only when the illness was severe. We suspect low lipid intake and defective synthesis of PL-protein in liver disease to be the possi-

ble mechanisms in production of the deficit in high density components of this system.

A regular result of taking a lipid-rich meal is the expansion of the size of this system of lipoproteins so that within three or four hours the total concentration of phospholipids assigned to this system commonly increases by as much as one-third.[3] After three or four days without any food the system commonly shrinks by one-third or more, without showing any marked distortion in pattern of distribution.[4]

The marked change in distribution of phospholipid in the centrifugate of serum which has been delipidated by repeated quick freezing in the presence

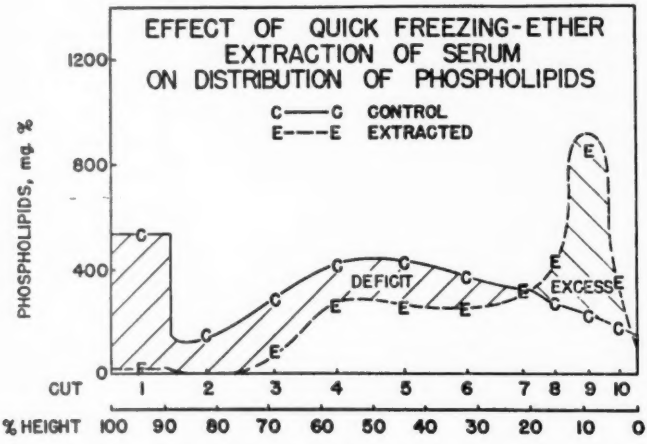

FIG. 7. Curves of distribution of phospholipid in two centrifugates of the same sample of serum. C, control untreated sample. E, sample delipidated by repeated freezing in the presence of ether before centrifugation.

of ether gives direct evidence that lipid load may influence localization of lipoproteins in centrifugates. In Figure 7 are shown two curves of distribution of phospholipid obtained from the study of a single specimen of serum, one portion of which had been delipidated before centrifugation by repeated quick freezing and thawing in the presence of ether according to the method of McFarlane.[5] This method removes nearly all of the cholesterol and neutral fat but only a small proportion of the phospholipid. The centrifugate of the treated serum shows a peak of phospholipid near the bottom of the tube and an absence of phospholipid in the top samples. We interpret these results as indicating that removal of neutral fat and cholesterol from lipoprotein complexes changes the sedimentation characteristics of some of the lipoprotein which retain the phospholipid so that there is a larger concentration of such lipoproteins in centrifugate of high density. The pattern of

distribution of phospholipid after delipidation in some respects represents a distortion opposite to that seen in acute hepatitis. We look upon the hepatitis pattern as a manifestation of overloading and that of delipidation as underloading of lipoproteins with lipid and suspect that size of fat load may be especially important in modifying localization of lipoproteins in the column. We have no means of differentiating fat carried by proteins of the different systems except in the top sample where fat is systematically related to other lipids.

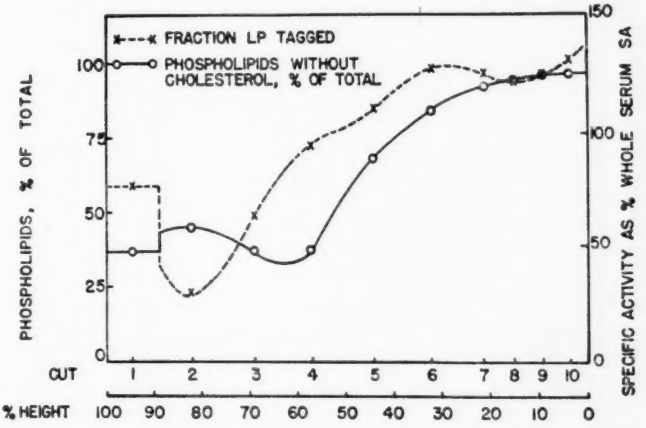

FIG. 8. The dotted line represents the mean specific activity of lipid phosphorus relative to whole serum specific activity in 13 centrifugates of six individuals, during the first two days after administration of radioactive phosphorus. Two individuals received therapeutic doses of P^{32} for polycythemia vera. The others received labeling doses. The solid line represents the proportion of phospholipid in each level which is assigned to the PL-protein system.

Our observations on tagging lipid phosphorus with P^{32} have shown that there are wide and systematic differences in the degree of tagging according to level in centrifugate.[9] The mean distribution of radioactive phosphorus expressed as specific activity of lipid phosphorus relative to specific activity of the whole serum in 10 centrifugates of six subjects during the first 48 hours after administration of isotope as inorganic phosphate is shown graphically in Figure 8. Another curve in this graph shows the proportion at different levels in the column of phospholipid which is assigned to the system without cholesterol. Below the two top samples the highest degree of labeling of lipid phosphorus occurred where the highest proportion of phospholipids without cholesterol was found. In a number of observations the curve showing specific activity in the centrifugate showed a peak of impressive height, sometimes localized to a small portion of the

column, and this peak was always found in that portion of the centrifugate which contained insignificant concentrations of the cholesterol-containing system. The top, or cream-layer-containing sample, seems to represent a special case and we have some evidence suggesting that in this sample the PL-C-protein system may contain a greater proportion of the P^{32} than does the system without cholesterol. The number two sample contains such small proportions of the total phospholipid that we can ignore the findings there. We interpret the data represented by these two curves as indicating that the mean rate of turnover of phospholipid in the system without cholesterol is probably higher than in the system with cholesterol.

The wide distribution of the system of lipoproteins which contains phospholipids but not free cholesterol is proof for inhomogeneity of complexes which contain these lipoproteins. Some components appear to be among the densest of plasma proteins while others which accumulate in the top sample will float on physiologic saline solution. We believe that the proportion of fat in the lipoprotein-fat complex is an important variable and is partly responsible for inhomogeneity. Other factors are no doubt important. Not only are the complexes of this system inhomogeneous in their sedimentation characteristics, and therefore structure, but differences in labeling with P^{32} at different levels in the column indicate differences in physiological activity. When the tagging of lipid phosphorus was followed in the same individual at appropriate intervals in the bottom four samples of centrifugate where PL-proteins are found with only a slight admixture of cholesterol containing lipoproteins, we have found such fluctuations in degree of tagging as to indicate that there are in that portion of dense centrifugate three imperfectly differentiated layers of phospholipid proteins, each made up of particles of distinctive sedimentation characteristics and each manifesting independent physiological behavior.[9] One possible basis for heterogeneity within a system of PL-proteins is that different components may contain only one kind of phospholipid or characteristic combinations of two or more. If such is the case, the functions of the particular protein would no doubt differ according to the kind of phospholipid which it contained.

It is our hope that eventually we may be able to interpret data such as are presented in this paper in terms of exact physiological mechanism. It would be premature to attempt any systematic interpretations at present. For example, the importance of the PL-protein system in meeting the sudden increase in burden on the lipid transport system following the ingestion of a lipid-rich meal seems clearly indicated, but there is as yet insufficient information for outlining any exact physiological mechanism. We have constructed some working hypotheses which we mention to illustrate some of the possible uses of our data. It is an attractive possibility that the lipoproteins without cholesterol may have a special function in the transport of fat to the liver. The alimentary lipemia data, for example, suggest

that this system functions in its transportation of fat from the alimentary tract. We wonder if the PL-C-protein system, in contrast, may have a special function in transporting fat from within liver cells to destinations outside the liver.

Summary

1. Some descriptive data are presented and discussed which deal with a system of lipoproteins containing phospholipid and believed to carry fat but which are devoid of free cholesterol and contain little or no cholesterol ester as well. The data selected were derived from multiple analyses of ten samples of centrifugate for each serum after application of standardized technique of quantity ultracentrifugation.

2. Evidence is reviewed for the general concept that the two forms of cholesterol and phospholipid enter into lipoprotein molecules in stoichiometric proportions and that fat is carried in variable proportions in lipoprotein-fat complexes.

3. Proof of the existence of lipoprotein devoid of free cholesterol is provided by the regular finding in bottom samples of centrifugate of untreated whole serum of nearly maximum concentrations of phospholipid, moderate concentration of fat and zero concentration of free cholesterol.

4. At levels where significant concentration of free cholesterol occurs, phospholipid was assigned to the cholesterol-containing system by multiplying free cholesterol concentration by a constant, and to the system without cholesterol by subtraction from total phopholipid estimated at that level. Concentration of the whole system in serum was calculated from data for the ten fractions of centrifugate. The constants were taken from formulae for lines of regression which described the free cholesterol-phospholipid relationship in normal centrifugate at different levels in the column.

5. As calculated from data for centrifugates of 18 healthy young men, the mean concentration of phospholipid with cholesterol was found to be 80 milligrams per cent (S.D. 21.1) and that without free cholesterol, 150 milligrams per cent (S.D. 16.4).

6. When centrifugates from a group of 47 patients with miscellaneous diseases were studied, the concentration of phospholipid with cholesterol showed high variability with tendency to high values while that in the system without free cholesterol showed small variability and tendency to low values.

7. The distribution of phospholipid without free cholesterol, according to sedimentation characteristics of the components, showed a high degree of constancy in health. A hypothetical plane across the centrifuge tube which divided phospholipid without free cholesterol into two equal compartments was found to have an average level of 1.027 specific gravity (S.D. 0.003).

8. The distortion of patterns of distribution in centrifugates of components of the system without free cholesterol is illustrated by typical examples drawn from study of acute liver disease and alimentary lipemia. The interpretation is offered that increased proportion of fat in lipoprotein-fat complexes may explain increase in proportion of low density complexes. The effect of *in vitro* delipidation of serum upon the distribution of phospholipid is also described.

9. Some features of the distribution of radioactivity in centrifugates after the injection of P^{32} as inorganic phosphate are described and interpreted as evidence that the mean rate of turnover of phospholipid without free cholesterol may be greater than that in the system of phospholipid-cholesterol-proteins.

Conclusions

The following hypotheses concerning the system of lipoproteins without free cholesterol are offered: Because of a tendency toward constancy of concentration of this system as a whole and also of its several components, together with evidence for a high level of physiological activity, we suggest that the parts of this system are integrated in a common function under the control of highly efficient regulatory mechanism. The function for which there is best evidence is transport of fat and fatty acids. The proportion of fat in a lipoprotein-fat complex may explain in part wide differences in localization in centrifugate of a given lipoprotein. The transport functions of this system may be of major importance in energy metabolism. Study of deviations from normal size of system or distribution of components under dietary stress or because of disease may contribute to a better understanding of normal and pathological physiology of lipids.

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CORTICOTROPIN AND CORTISONE IN DISEASES OF THE NERVOUS SYSTEM*

H. HOUSTON MERRITT†

When corticotropin and cortisone became generally available in 1950 they were tested as therapeutic agents in a wide variety of morbid conditions without much consideration as to rationale. It was only natural that these hormones should be tried in a number of diseases of the nervous system in which the existing therapeutic agents are inadequate. It is the purpose of this article to review critically the results reported to date and to compare

TABLE 1

TREATMENT OF PROGRESSIVE MUSCULAR DYSTROPHY WITH CORTICOTROPIN OR CORTISONE

Authors	*Clinical type of dystrophy*	*Number of cases*	*Results*
Milhorat[15]	Facioscapulohumeral	3	No change
Glaser and Merritt[8]	Pseudohypertrophic	3	No change
	"Menopausal type"	3*	No change in 2 Equivocal improvement in 1
Shy and McEachern[25]	Juvenile type	3	No change
	"Menopausal type"	8	7 greatly improved 1 unchanged

* Not previously reported.

them to those obtained by Dr. Gilbert Glaser and the author[8] at the Neurological Institute of the Presbyterian Hospital, in the hope that the benefits and limitations of these compounds may be determined.

A. DISEASES OF THE NEUROMUSCULAR APPARATUS

1. *Progressive muscular dystrophy.* Twenty cases of progressive muscular dystrophy have been treated (Table 1). In nine cases of the juvenile or facioscapulohumeral type, there was no appreciable effect on the strength of the patient or the course of the disease. In contrast to the poor results in

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the juvenile type of dystrophy, Shy and McEachern[25] reported excellent results in the treatment of patients with a type of dystrophy which they characterize as "menopausal dystrophy" because of the relatively late onset of the symptoms. Good to excellent results were obtained in seven of eight patients with this type of muscular dystrophy. Continual administration of corticotropin or cortisone was necessary to maintain improvement. We have treated three cases of muscular dystrophy in females, in which the onset of symptoms was in middle life, without any significant change in the muscular status of the patients.

2. *Myotonia atrophica and myotonia congenita.* Fifteen cases with myotonic atrophica have been treated (Table 2). Negative results were reported

TABLE 2

THE EFFECTS OF ACTH AND CORTISONE IN THE TREATMENT OF MYOTONIA ATROPHICA

Authors	*Type of cases*	*Number of cases*	*Results*
Milhorat[15]	Myotonia atrophica	2	No change
Shy and McEachern[25]	Myotonia atrophica	3	Myotonia decreased No change in muscle strength
	Myotonia congenita	1	Myotonia disappeared
Reese and Peters[18]	Myotonia atrophica	7	Myotonia decreased Slight or no improvement in muscular strength
Glaser and Merritt[8]	Myotonia atrophica	3	No change

by Milhorat[15] (two cases) and Glaser and Merritt[8] (three cases). Moderate or great improvement in the myotonic symptoms were reported by Shy and McEachern[25] in three cases and by Reese and Peters[18] in seven patients without any significant improvement in the muscular strength of any of these ten patients. Shy and McEachern[25] also report complete abolition of the myotonia in one patient with myotonia congenita who was treated with corticotropin. The myotonia was greatly decreased but not abolished when cortisone was administered by mouth.

3. *Myasthenia gravis.* The literature contains reports on 36 patients with myasthenia gravis (Table 3) who have been treated with corticotropin or cortisone. In a small percentage of the cases, administration of these compounds is not accompanied by any significant change in the clinical condition of the patient. In the majority, however, there is a temporary exacerba-

tion of the symptoms during the period of therapy, followed by a remission of symptoms of a slight, moderate, or great degree. The remission is rarely complete, consisting mainly of a decrease in the daily prostigmine requirement. These remissions are temporary, lasting only a few weeks to several months. Repeat treatment is usually followed by recurrence of the remission. Several patients have died in the course of the treatment or within a few weeks after the treatment had been completed.

4. *Amyotrophic lateral sclerosis.* Thirteen patients with amyotrophic lateral sclerosis have been treated with corticotropin or cortisone. No

TABLE 3

THE EFFECT OF ACTH AND CORTISONE ON MYASTHENIA GRAVIS

Author	*Number of cases*	*Results*
Torda and Wolff[29]	15	Significant partial remission in 10 Death on third day of treatment in 1
Shapiro[24]	3	Partial remission in 1 No change in 1 Downhill course with death in 1
Schlezinger[20]	11	Partial remission in all 11 One case died in acute relapse
Millikan and Eaton[17]	5	No change in 1 Partial remission in 3 Death two weeks after treatment in 1
Soffer[26]	1	Partial remission in 1*
Glaser and Merritt[8]	1	Partial remission with death six weeks later

* Accompanied by decrease in size of thymic tumor.

appreciable benefit was noted in three patients treated by Shy and McEachern,[25] one by Millikan and Eaton,[16] two by Jacobson,[11] and seven by Glaser and Merritt.[8] An increase in muscular weakness was noted in two of the seven patients in the last series.

B. PERIPHERAL NEURITIS

Eight patients with multiple peripheral neuritides (Table 4) have been treated with corticotropin or cortisone. No improvement was noted by Shy and McEachern[25] in three patients with peripheral neuritis of unknown cause. Improvement in muscular strength and apparent shortening of the clinical course in patients with polyneuritis of the type classified as the Guillain-Barré syndrome was reported in one case by Stillman and Ganong[28] and in a second case by Seltzer, Lichty, and Conn.[23] Negative

results were obtained by us in three cases with polyneuritis of unknown cause, two of which were diagnosed as conforming to the Guillain-Barré type.

C. Multiple Sclerosis

Thirty-three patients with multiple sclerosis were treated with corticotropin by Glaser and Merritt.* Twelve (36 per cent) showed changes suggestive of significant general improvement. The specific symptom complexes most favorably affected were the spastic-paretic, cerebellar, or ataxic and acute visual. In most instances improvement was transient, appearing only during the course of treatment and lasting for only a week or two after the treatment had been discontinued. A small series of cases is being kept on

Table 4

The Results of Treatment of Polyneuritis with Corticotropin or Cortisone

Authors	*Type of polyneuritis*	*Number of cases*	*Results*
Shy and McEachern[26]	"Unknown cause"	3	No improvement
Seltzer, *et al.*[23]	"Guillain-Barré syndrome"	1	Rapid improvement
Stillman, *et al.*[24]	"Guillain-Barré syndrome"	1	Rapid improvement with relapse and subsequent improvement
Glaser and Merritt[8,9]	"Unknown cause"	1	No improvement
	"Guillain-Barré syndrome"	2	No improvement

* Not previously reported.

maintenance dosages of corticotropin or cortisone in an attempt to determine whether long-term administration of these compounds will prevent acute relapses of the diseases. The number of cases so treated and the period of observation are inadequate for any conclusions.

A number of cases of multiple sclerosis have been treated by Forsham, Thorn, and Alexander[5] and by Fog[4] with results similar to those reported above.

Eighteen patients with acute retrobulbar neuritis, occurring as a symptom in the course of multiple sclerosis or as an isolated phenomenon, have been treated by McLean[14] and by Gammon, Scheie, Ojers, Tyner, and King.* McLean[14] reported rapid improvement in visual acuity and decrease in size of scotomata in two patients. Gammon and his associates[6] reported that lessening of pain, decrease of edema of the optic nerve, and increase in visual acuity occurred one to ten days after the initiation of treatment. The final

results in regard to vision were: Complete or almost complete return, seven; partial return, four; no useful vision, five. This series is too small to permit one to draw any conclusions, but it is our impression that the results reported are not significantly different from those obtained with no treatment or with other forms of treatment.

Although transient improvement may be noted in individual symptoms in patients with multiple sclerosis who are treated with corticotropin or cortisone, it can hardly be claimed that the treatment of this disease with these compounds is on the whole satisfactory.

D. Epilepsy

It was noted by Hoefer and Glaser[9] that the administration of corticotropin may be accompanied by changes in the electroencephalogram. These changes were not of the nature to suggest that this compound would be of

Table 5

Various Neurological Diseases Treated by Corticotropin and Cortisone
(Compiled from literature)

Disease	*Number of patients*	*Effect*
Acute anterior poliomyelitis[2, 16]	37	No effect on clinical course
Sydenham's chorea[1, 6, 22]	5	No improvement 3 Improved 2
Infectious mononucleosis[10]	1	No effect on clinical course
Tay-Sach's disease[7]	1	No effect on clinical course
Wilson's disease[8]	2	No effect on clinical course
Parkinsonism[9]	3	No effect on clinical course

value in the treatment of patients with epilepsy. Furthermore, isolated convulsive seizures or status epilepticus have been reported in the course of treatment of various conditions with corticotropin or cortisone by Soffer, *et al.*,[26a] Russell, Haserick, and Zucker,[19] Stephen and Noad,[27] and by Dorfman, Apter, Smull, Bergenstal, and Richter.[3] In some of these cases the exact relationship of the seizures to the administration of the hormones is not clear, since it is known that convulsive seizures are not uncommon in several of the conditions (lupus erythematosus, periarteritis nodosa) which were the subject of these reports, but in other cases it seemed obvious that the convulsions were related to the administration of the hormones.[18] Russell, Haserick, and Zucker,[19] however, reported that there was improvement in the epileptiform seizures associated with lupus erythematosus while under ACTH treatment, although some of their cases had convulsions for the first time coincidental with the administration of the hormone.

Klein and Livingston[19] reported improvement in the seizure incidence and in the electroencephalograms of four of six children with epilepsy. In the three patients treated by Glaser and Merritt,[8] there was no change in the frequency of seizures. The electroencephalogram became more abnormal in one patient.

Various Conditions

Thirty-five patients with *acute anterior poliomyelitis* (Table 5) were treated by Coriell, Siegel, Cook, Murphy, and Stokes.[3] ACTH was given to the patients in the early stage of the disease and the study was well controlled. The treatment had no effect on the course of the disease or on the incidence of paralysis. Similar results were reported by Millikan and Eaton[16] in two cases. Experimental work in animals has shown that the administration of these hormones may increase the susceptibility to infection with the poliomyelitis virus.[21]

Two cases of *Sydenham's chorea* were treated with cortisone by Aronson, Douglas, and Lewis[1] and one by Glaser and Merritt[8] without benefit. Schwarz,[22] however, reported improvement in two patients with the severe form of the disease.

One patient with *infectious mononucleosis* with cerebral involvement was treated with ACTH and aureomycin by Huber, Bailey, Campbell, and Mathieson[10] with no appreciable effect on the course of the disease.

One case of *cerebromacular degeneration* (Tay-Sach's disease) was treated by Gitman, Brusilow, and Greenblatt[7] with ACTH. The treatment did not retard the progressive course of the disease.

An editorial in the United States Armed Forces Medical Journal[20] reported that ACTH had been used with benefit in a few cases of *post-vaccinal encephalitis.* No details as to symptoms, dosage, or effects of the therapy were given.

Two patients with *Wilson's disease* and three patients with *Parkinsonism* were treated by Glaser and Merritt[8] without any appreciable benefit.

Discussion

Corticotropin and cortisone have been used in a variety of neurological diseases but have not proven to be of curative value in any of them. Temporary remission of symptoms occurred in many of the patients with myasthenia gravis. There was some improvement in a few cases of multiple sclerosis and in some patients with "menopausal dystrophy." The last observation has been made by only one group of investigators and has as yet not been confirmed by others.

Conclusions

Corticotropin and cortisone are of little or no value in the treatment of the majority of neurological diseases. In a few diseases there may be a

slight degree of improvement. Short-term administration of these hormones in myasthenia gravis may be followed by temporary partial remissions.

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EXPERIMENTAL PRODUCTION OF GYNECOMASTIA WITH CHORIONIC GONADOTROPIN*

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The male mammary glands often undergo hyperplasia in the presence of tumors associated with a high urinary excretion of chorionic gonadotropin. Included in this group are the chorioepitheliomas, both testicular[1-13] and extragenital,[14-23] and, more rarely, teratomas[24, 25] and seminomas[24, 26] of the testis. Although the histological and clinical features differ in each of these tumors, there is reason to believe that all contain chorionic elements when accompanied by chorionic gonadotropin in the urine.[1, 27]

Less commonly gynecomastia occurs in association with non-choriogenic tumors of the testis. These comprise a heterogeneous group with respect to their hormonal excretion patterns, but in general fall into one of two categories: (i) those in which the gynecomastia is probably a non-specific effect related to loss of testicular tissue by tumor invasion, radiation therapy, or surgical extirpation,[28] and in which the excretion of androgen is low and of follicle-stimulating gonadotropic hormone (FSH) high,[29] and (ii) those in which the gynecomastia appears to be more directly related to hormonal stimulation by the tumor itself, as in Sertoli-cell[30] and aberrant adrenocortical[31] tumors. In these the excretion of estrogen is increased, and since removal of the tumor results in both a reduction in urinary estrogen and a regression in the gynecomastia, it is highly probable that the tumor is the source of the estrogen and that the latter is an important factor in stimulating mammary growth. Gynecomastia has also been reported as a rare complication in interstitial- or Leydig-cell tumors,[32, 33] but the histological diagnosis in such cases has been questioned.[34] As a rule these tumors produce virilization in children, but exert no hormonal effects in adults.[33] On the basis of a study of dog tumors, Huggins and Moulder[34] have presented evidence to show that those testicular tumors associated with gynecomastia and previously reported as interstitial-cell tumors were probably incorrectly interpreted examples of Sertoli-cell tumors. However, in one of these cases, that of Hunt and Budd,[32] the possibility of chorionic elements in the tumor does not appear to have been completely excluded, since the urine contained

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a large amount of luteinizing gonadotropin, presumably of the chorionic type.

The contributions of Hamburger and his associates[17, 29, 35] have shed considerable light on the relationships between testicular tumors and the urinary gonadotropins. These investigators were the first to point out the necessity for distinguishing between the excretion of luteinizing chorionic gonadotropin derived from certain types of testicular tumor tissue, and the excretion of follicle-stimulating gonadotropin originating in the anterior lobe of the pituitary in response to a decrease in androgen secretion, related to non-specific testicular injury. They clearly demonstrated that only in the case of the choriogenic tumors is extirpation followed by a decrease in gonadotropin excretion. Moreover, they attempted to correlate the type of gonadotropin excreted with the histological features and clinical course of the tumors they studied, and concluded that the excretion of chorionic gonadotropin was characteristic of radioresistant, mixed epitheliomas, a group of tumors including chorioepitheliomas and embryonal adenocarcinomas,[27] and that it denoted a bad prognosis, while the excretion of FSH in increased amounts was characteristic of radiosensitive seminomas and indicative of a more favorable outlook. Further studies by Furuhjelm[36] and Twombly[27] have confirmed the different sites of origin and significance of the two types of gonadotropin, but have not corroborated the close correlation between the type of gonadotropin present in the urine and the histological features of the tumor reported by Hamburger. However, it is well recognized that the structure of the testicular tumors, and especially of their metastases, is often not uniform, and it is highly probable that chorionic elements are always present when the urine contains chorionic gonadotropin.[1, 27]

Ever since Cooke[2] first called attention to the occurrence of gynecomastia in chorioepithelioma in 1915, the subject of its pathogenesis has been a matter of speculation and debate. Unfortunately, not even the great strides made in endocrinology since then have added materially to Cooke's original hypothesis that the gynecomastia is probably the result of stimulation by a substance present both in chorioepitheliomatous tissue and the normal placenta. It is now known, of course, that several analogous, if not identical, hormones can be isolated from each of these tissues, but whether, in the case of testicular chorioepithelioma, any one of these hormones stimulates the mammary gland directly or exerts its effects indirectly by activating other endocrine glands, is still unknown. It is reasonably certain, however, from cases like Warthin's,[37] in which the gynecomastia subsided after the tumor had been removed and recurred when metastases made their appearance, and from cases of extragenital chorioepithelioma with gynecomastia,[24–33] that the gynecomastia is related to the humoral activity of the tumor and not to the coincidental testicular atrophy which is often present.

Most authors[13, 20, 28] ascribe the gynecomastia to a direct estrogen effect, and certainly the frequency with which hyperestrinuria has been demonstrated[13, 17–19] and the similarity between the type of mammary duct hyperplasia found in chorioepithelioma and that produced by estrogen[38] appear to support this hypothesis. However, there is no unanimity of opinion regarding the origin of this estrogen. Many[19, 20, 27] consider it to be a secretory product of the tumor itself because of the high estrogen levels found in some chorioepitheliomas.[14, 15] Others regard the testis as the source of estrogen, basing this opinion on the experiments of Geschickter and his associates,[38] who showed that chorionic gonadotropin derived from pregnancy urine produced mammary growth in intact male monkeys, but not in castrates, and on the frequent occurrence of interstitial-cell hyperplasia in the unaffected testis in chorioepithelioma.[13, 19, 23, 24] While the testis may play a rôle in the pathogenesis of gynecomastia in chorioepithelioma, the hyperplastic interstitial cells can hardly be taken as evidence of increased estrogen production in the light of present knowledge concerning their androgen-producing function. Finally, it has been suggested[13, 29] that the adrenal cortex may be the source of estrogen under the influence of chorionic gonadotropin stimulation. Certainly there is evidence that some adrenocortical derivatives are estrogenic,[39] and that the adrenal cortex may be stimulated by chorionic gonadotropin.[40] Moreover, Ferguson[24] has reported the occurrence of swelling and hyperemia of the adrenals in cases of teratoma of the testis with elevated urinary gonadotropins. However, this has not been found with any degree of regularity,[1, 2, 4, 9, 11, 14, 15, 21, 22] nor can the possibility be excluded that the adrenocortical hyperplasia in such cases is the result rather than the cause of hyperestrinemia, an effect well recognized in animals.[41, 42]

The pituitary is enlarged in many cases of chorioepithelioma and shows changes in the cellular pattern of its anterior lobe resembling those of pregnancy.[2, 3, 5, 7, 8, 10–13, 18, 23, 24] This has led Gilbert[5] and others to suggest that the development of gynecomastia may be a pituitary effect. Although this hypothesis is consistent with the mammogen theory of breast development once held by Turner and his associates,[43–45] more recent work[46–49] indicates that the pituitary may exert a potentiating or sensitizing effect, but that it does not promote mammary growth directly.

In a significant number of chorioepitheliomas the mammary glands go on to alveolar development[5, 10, 12] and the secretion of a colostrum-like material,[2, 4, 5] features not seen ordinarily after estrogen administration, but highly suggestive of a progesterone effect. The possibility that progesterone may be implicated has been considered, and, indeed, pregnandiol has been recovered from the urine in several instances.[13, 19, 27] However, it is unlikely that progesterone is the primary factor, since, except in the mouse,[48] it does not produce proliferation of the duct system.[50]

It is obvious from the preceding discussion that the pathogenesis of choriogenic gynecomastia is still far from well understood, and that further

hormone assays and morphological studies of the endocrine system in such cases are not likely to add materially to what is already known. Only the experimental approach appears to offer any hope of solving this problem, and since the response of the mammary gland to hormonal stimuli varies in different species it would be highly desirable to conduct these studies in man. Recently we were afforded an opportunity to carry out such experiments and these are the subject of the present report. They were undertaken following the chance observation, made during the course of some studies of endocrine function in a patient with Laennec's cirrhosis, that massive doses of chorionic gonadotropin produced gynecomastia. Similar effects were observed in a second case of cirrhosis and in two carefully selected controls, one with marked congenital atrophy or aplasia of the testis, and the other with marked pituitary deficiency, but not in two other subjects. Parallel studies of hormone excretion and of the histological changes in the testes and mammary glands were carried out. While the results of these experiments fail to elucidate the pathogenesis of choriogenic gynecomastia, they do clarify several disputed issues, and, more important, point the way for further similar studies.

Experimental Subjects

Seven subjects were studied in the manner to be described—six males and a female control. Cases 1 and 2 were young cirrhotics who developed gynecomastia following the administration of chorionic gonadotropin. Case 3, a young cirrhotic who also had congenital (?) testicular atrophy, was selected for study to determine whether the testes were essential for this action of chorionic gonadotropin. Since gynecomastia failed to develop in this subject, case 4, a young man with congenital (?) atrophy or aplasia of the testes but without evidence of liver disease, was investigated as a control for case 3, and also to determine whether the presence of liver disease was essential for the mammary effect of chorionic gonadotropin. Case 5, a young man with severe pituitary deficiency, was selected for study to determine whether the effects of chorionic gonadotropin were mediated through the pituitary. Case 6 was a presumably normal male control with respect to his endocrine system and liver; however, biopsy of the testis revealed some degree of hypoplasia. Case 7 was a middle-aged female with cirrhosis who served as a control for the hormone excretion studies in the six male subjects investigated.

Case 1. Laennec's cirrhosis. E.R., V-47 149, a 32-year-old white, married male, had a history of chronic alcoholism and poor diet for an indeterminate number of years. Cirrhosis was discovered three years before the present study, following an hematemesis. Hematemesis recurred a year later and again shortly before admission to the hospital. Loss of libido had been noted for six to eight months.

The patient was well developed and exhibited no signs of malnutrition. There were several large spider nevi, but no icterus, ascites, edema, or visible portal venous col-

lateral circulation. Both the liver and spleen were moderately enlarged and firm. The testes were soft but were not decreased in size. The penis and prostate were normal in size and there was a normal distribution of hair.

Biopsy of the liver revealed an extensive cirrhosis with marked fatty infiltration, and chemical studies indicated moderate impairment of function (Table 1). Esophageal varices could not be demonstrated by x-ray. There was moderate anemia due to hemorrhage, but this was promptly corrected by transfusion.

Case 2. Laennec's cirrhosis. V.V., V-47 343, a 38-year-old, white, single male, had a history of chronic alcoholism and a poor diet for six years. He was admitted to the hospital because of jaundice and recurrent epistaxis of one month's duration. There was a history suggestive of an attack of infectious hepatitis six years earlier. No loss of libido had been noted.

The patient was well developed and exhibited moderate obesity, slight icterus, numerous spider nevi, and marked hepatosplenomegaly, but no ascites, edema, testicular atrophy, or loss of hair.

Biopsy of the liver revealed severe cirrhosis with marked fatty infiltration, and liver function was markedly impaired (Table 1). No esophageal varices could be demonstrated by x-ray.

Case 3. Laennec's cirrhosis and congenital (?) testicular atrophy. J.M., N-B 79 312, a 37-year-old white, single male, was admitted to the hospital because of anemia, anorexia, weight loss, and dyspnea of several months' duration. Alcohol consumption had been high and the diet inadequate for an indeterminate period. The testes and penis had been very small since childhood, but the patient claimed that libido and potency had been normal until one year previously when both were lost. There was a history of mumps in childhood, but none of orchitis or testicular injury.

The patient looked chronically ill and exhibited marked pallor, slight icterus, and signs of moderate malnutrition. A small amount of ascitic fluid was present, but there were no signs of portal collateral circulation, edema, or spider nevi. The liver and spleen were moderately enlarged and firm. The penis and scrotum were markedly underdeveloped, and the testes were very small and soft. The prostate was small and firm. The voice was rather high-pitched and the general configuration of the body was feminine, but the pubic and axillary hair distribution was that of a normal adult male.

Biopsy of the liver revealed a severe cirrhosis with marked hemosiderosis and fatty infiltration, and chemical studies indicated moderate impairment of function (Table 1). Hematological studies revealed a moderately severe macrocytic hyperchromic anemia, but the gastric juice contained free HCl, the bone marrow showed normoblastic hyperplasia, and there was no increase in erythrocyte fragility. A glucose tolerance test was normal. Neither the nature of the anemia nor the significance of the hemosiderosis was adequately explained, although the former improved following transfusions and improvement in the diet.

Case 4. Congenital (?) atrophy or aplasia of the testes. W.S., V-47 981, a 34-year-old married, white male, was admitted to the hospital for treatment of a peptic ulcer of two years' duration. The testes had always been very small, but physical and sexual development had been normal in other respects. The patient began to shave at the age of seventeen and regarded his libido and potency as normal. He had been married for four years and enjoyed a normal sex life, although he had proved to be sterile. There was no history of mumps orchitis, testicular injury, or serious illness in the past. There had been a weight gain of 70 pounds in the preceding seven years.

The patient was a tall, muscular, somewhat obese young man who measured six feet in height and weighed 200 pounds. There was a heavy beard and a moderate growth of hair in the axillary and pubic areas and over the trunk. The voice was moderately

TABLE I

CLINICAL AND LABORATORY FEATURES IN THE FOUR CASES OF CIRRHOSIS INVESTIGATED

	Clinical findings											Liver function tests												
													Serum bilirubin						Serum proteins				Serum cholesterol		
Case	Jaundice	Ascites	Edema	Wt. loss	Hepatomegaly	Splenomegaly	Esoph. varices	Spider nevi	Testicular atrophy	loss libido	Hair loss	Day of expt.†	1 min.	Total	BSP	Ceph. floc.	Thymol turb.	Alk. phosp.	Total	Alb.	Glob.	Prothrombin	Total	Free	Per cent Free
													mg. per cent		per cent		units	B.u.	gm. per cent			per cent	mg. per cent		
1. E.R.	0	0	0	0	1F	1F	+*	++	+	++	0	—23	0.3	1.3	29	3+	5.3	4.1	5.1			53	204		
												—10	0.2	0.7	8	4+	4.0	3.4	4.7			50	170		
												11	0.2	0.9	6	2+	1.0	3.2	5.9			48	198		
												34	0.2	0.6	9	2+	3.5	2.4	5.5			63	134		
												57	0.2	0.6	5	2+	1.3	3.3	7.4			60	124		
												74	0.2	0.4	7	0	1.0	3.5	7.4				129		
2. V.V.	++	0	0	0	5F	2F	0	+++	0	0	0	—7	4.6	6.8	62	4+	4.0	4.9	6.3			43	237		
												14	1.4	3.0	43	2+	1.5	4.2	5.5			45	191		
												32	1.2	2.6	42	4+	1.5	2.5	5.9			59	198		
												54	0.6	2.0	31	3+	2.3	2.3	6.7			68	198		
												74	0.6	1.2	25	2+	1.5	2.1	6.3			59	154		
												80	0.2	0.7	21	4+	1.5	4.2	6.7			63	180		
3. J.M.	+	+	0	++	3F	1F	0	0	+++	++	0	—5	0.8	1.7	28	1+	2.0	11.3	5.37	2.24	3.13		176	71	40
												—1	0.9	1.0	31	0	1.5	9.7							
												6	0.6	1.7	26	0	2.0	7.5	6.25	2.61	3.91				
												16	0.4	1.2	38	0	4.5	5.6	6.71	3.03					
												24	0.3	0.4	20	2+	2.0	5.6	7.29	3.41	3.88		179	60	30
												40	0.2	0.8	13	0	1.0	4.9	6.87	3.60	3.27				
7. T.F.	++	++	++		0	9F	0	0	+	.....	?	0	—3	1.9	4.6	38	4+	3.0	7.6	5.73	2.04	3.69	60	255	129	50
												4	2.0	3.8		3+	2.0								
												11	1.5	3.1	35	3+	3.0	7.7	6.19	2.40	3.79	62	257	98	38
												18	0.9	1.94	26	3+	5.5	5.6							
												25	0.6	1.6	22	3+	5.5	5.8	6.29	2.66	3.77		236	96	40

* Hematemesis, but varices not demonstrated by x-ray.

† Number of days from onset of chorionic gonadotropin administration.

deep and the penis appeared normal, measuring 8 cm. in length, but the scrotum was unusually small and contained 2 small ovoid masses measuring 2 x 1 x 1 cm. each. These were assumed to be testes, but on two occasions when they were biopsied only epididymal tissue was obtained. The biopsies were carried out on opposite sides by two different surgeons, the second being a urological specialist who was aware of the previous failure to obtain testicular tissue. It is highly probable, therefore, that there was little or no testicular tissue present. In keeping with this assumption was the observation that a sample of semen contained no spermatozoa. The prostate appeared to be of normal size. The remainder of the examination revealed no other abnormalities.

Laboratory studies, including tests of liver function, were all within normal limits, except for the results of the urinary hormone assays which will be discussed elsewhere.

It was difficult to classify the nature of the testicular disorder in this case since the entire scrotal contents were not available for study, but in many ways it appeared unique. In some respects it was reminiscent of the prepubertal functional castration described by Heller, Nelson, and Roth,[51] but the absence of overt eunuchoidism appeared to exclude this possibility. Similarly, the syndromes of Klinefelter[52] and del Costillo[53] were considered, but the complete lack of testicular elements did not appear compatible. Conceivably this was an example of testicular aplasia with maintenance of the secondary sex characters by the adrenal cortex, as has been described in rare cases of castration.[54]

Case 5. Pituitary deficiency. J.R., V-48 935, a 37-year-old single, white male, was admitted to the hospital because of attacks of headache and vomiting of several months' duration. One and one-half years previously he had suffered an attack of meningitis, and immediately following had developed typical symptoms of myxedema, loss of facial and body hair, and marked atrophy of the testes associated with complete loss of libido and potency. There was a history of chancre seven years previously, presumably cured following a six months' course of therapy.

The patient exhibited typical signs of severe myxedema. Facial and body hair was very sparse. The skin was coarse, pale and dry and had a fawn-colored pigmentation over the face. The testes were very small and soft and were associated with marked atrophy of the penis and prostate. The fundi, visual fields, and neurological examination revealed nothing of note. The blood pressure was low at all times, ranging between 80/52 and 98/64.

The basal metabolic rate and the serum precipitable iodine were low, the former ranging between —24 and —30 per cent, the latter between 4.1 and 2.7 gamma per cent. The results of the Kepler-Power test were equivocal with respect to adrenal function, but there was no fall in eosinophils following an injection of epinephrine. A single injection of ACTH failed to lower the eosinophil count in four hours, but multiple doses lowered the count slightly in 24 hours. The blood sugar and serum electrolytes were within normal limits. Intravenous glucose and insulin tolerance tests were likewise normal. X-rays of the skull revealed no abnormalities. X-ray of the chest showed the presence of calcified tubercles and hilar nodes. A tuberculin test (P.P.D. first strength) was positive. A histoplasmin skin test was also positive, but the complement fixation test for histoplasmosis was negative. Serological tests for syphilis were negative. The spinal fluid was not examined at this time, but subsequently showed a negative serological test for syphilis; cultures for bacteria and fungi, guinea-pig studies for tubercle bacilli, and inoculation of animals and eggs for viruses all failed to demonstrate any organisms.

There seems to be little doubt that this patient had pituitary deficiency manifested by myxedema, depressed gonadal function, and possibly mild Addison's disease. However, the nature of the pituitary lesion was not determined. The possibility of a tuberculoma was seriously considered, especially when the patient developed a second attack of

meningitis some time after these studies were completed. Since then his symptoms have been relieved by substitution therapy with thyroid extract and testosterone proprionate, but the diagnosis still remains in doubt.

Case 6. "Normal" male control. S.P., V-54 558, a 39-year-old white male, had been hospitalized for eight months because of multiple sclerosis of three years' duration at the time of these studies.

He was in a good state of nutrition and had a heavy beard and deep masculine voice. The external genitalia were above average in size, the testes measuring 4.3 x 2.2 x 2.7 and 4.7 x 2.4 x 2.9 cm. respectively. The remainder of the physical examination revealed no abnormalities other than signs of advanced multiple sclerosis.

Routine laboratory studies, including tests of liver function, yielded normal results.

Case 7. Female cirrhotic control. T.F., N-C 21 233, a 43-year-old white housewife, was admitted to the hospital because of ascites and jaundice of several weeks' duration. There was a history of alcoholism and poor diet for 15 years. A left oophorectomy had been performed at the age of 14, but the menstrual cycles had continued regularly until 14 months previously when they ceased abruptly.

The patient was moderately obese and did not appear ill. There was moderate icterus of the skin. One spider nevus was present on the neck. The liver was greatly enlarged and firm and was associated with moderate ascites and visible portal venous collateral circulation. The spleen, however, was not palpable. The ankles were moderately edematous.

Liver biopsy revealed moderately severe cirrhosis with marked fatty infiltration, and chemical studies indicated moderately severe impairment of liver function (Table 1). There was a moderate macrocytic anemia. No esophageal varices could be demonstrated by x-ray.

Methods

Chorionic gonadotropin administration. The preparation used ("A.P.L.," Ayerst, McKenna and Harrison, Limited*) was administered intramuscularly as indicated in Table 2. This material is extracted from human pregnancy urine by the method of Collip[55] and is said to consist almost exclusively of luteinizing gonadotropin, although negligible traces of FSH and prolactin may be present; the method of extraction is thought to preclude the presence of steroids.[56]

Liver function tests. The methods used in this laboratory have been described elsewhere.[57]

Urinary hormone assays. Twenty-four or 48-hour specimens of urine were obtained from the patients with special precautions to insure completeness of collection. No preservative was added, but the urine was kept refrigerated and processed within 24 hours after completion of the collection.

A full or half 24-hour specimen was extracted for assay of 17-ketosteroids, androgens, estrogens, and "estroids," as described by Salter, Humm, and Oesterling.[58]

17-ketosteroids were estimated in the crude neutral steroid extract by the method of Cahen and Salter.[59] Range of values for normal males 10 to 25 mg. per day, and for non-pregnant females 6 to 20 mg.

Androgen excretion was determined in aliquots of the crude neutral fraction by the bioassay method of Rakoff, Paschkis, and Cantarow.[60] Range of values for normal

* The authors are indebted to Dr. S. H. Jaros, Medical and Research Division, Ayerst, McKenna and Harrison, Limited, New York, New York, for generous supplies of the "A.P.L." used in this investigation.

males, 2 to 10 mg. of androsterone equivalent (20 to 100 international units of androgen) per day, and for non-pregnant females, 1 to 10 mg. of androsterone equivalent.

"Estroids" were determined colorimetrically by the modified Kober procedure described by Salter and his associates,[58, 61] using a purified phenolic fraction. Range of values for normal males, 20 to 80 micrograms of estrone equivalent per day and for non-pregnant females, 40 to 120 micrograms.

Estrogen excretion was also estimated biologically in the crude phenolic fraction by a modification of the Marrian and Parkes and Mather methods, as described by Humm, Munson and Salter.[61] Range of values for normal males 0.5 to 3.0 micrograms of

TABLE 2

RELATIONSHIP BETWEEN CHORIONIC GONADOTROPIN ADMINISTRATION AND DEVELOPMENT OF GYNECOMASTIA

	Gonadotropin administered			*Gynecomastia*			
					Appearance time		
Case	*Daily dose*	*Days*	*Total Dose*	*Side*	*Days*	*Gonadotropin*	*Maximum size*
	units		*units*			*units*	*cm.*
1	2,000	70	140,000	Left	34	68,000	2.5
2	2,000	80	160,000	Left	29	58,000	2.5
3	4,000	40	160,000	None			
4	2,143*	95	195,000	Left	53	115,000	4.0
				Right	95	195,000	4.0
5	4,000	45		Right	43	172,000	2.5
	10,000	34	510,000	Left	56	290,000	2.0
6	5,000	10					
	10,000	19	240,000	None			
7	2,000	30†	60,000	Female control: no change in breasts			

* 5,000 units 3 times a week.
† period of administration 32 days.

estrone equivalent (5 to 30 international units of estrogen) per day, and for non-pregnant females 1 to 6 micrograms of estrone equivalent.

It is recognized that the short period of acid hydrolysis of urine (7 minutes), which was chosen because it is desirable for the liberation of the neutral steroids from their conjugates, may have been inadequate to obtain a quantitative yield of the estrogens. It is assumed, nevertheless, that the estrogen values reported are valid for comparative purposes.

Gonadotropin (FSH) excretion was estimated by the method of Varney and Koch,[62] usually on a full 24-hour specimen of urine. Range of values for normal males, 10 to 40 mouse uterine units per day and for non-pregnant females, 10 to 50 mouse uterine units.

The normal values cited are based on the experiences of this laboratory. However, it should be emphasized that the values for women are based on more limited experience than those for men, and that a minority of subjects of both sexes yield data somewhat outside the ranges given.

Results

Mammary glands. Four of the six male subjects who were given chorionic gonadotropin developed typical gynecomastia (Table 2). These included two of the three patients with Laennec's cirrhosis (cases 1 and 2), the patient with congenital (?) atrophy or aplasia of the testes (case 4), and the patient with pituitary deficiency (case 5). Only the cirrhotic with congenital (?) atrophy of the testis (case 3) and the "normal" male control (case 6) failed to develop gynecomastia.

The first sign of gynecomastia appeared on the 29th to 53d day of gonadotropin therapy, or following the administration of a total dose of 58,000 to 172,000 international units. It is of interest that the gynecomastia was bilateral in only two of the four subjects (cases 4 and 5), and that in each instance the second mammary gland took considerably longer to undergo hyperplasia than the first, not appearing until the 95th and 56th day of treatment respectively, or following the administration of 195,000 and 290,000 international units. Since cases 1 and 2 received only 68,000 and 58,000 international units, respectively, it is highly probable that they too would have developed bilateral gynecomastia had gonadotropin therapy been continued longer. Similarly, the failure of gynecomastia to develop at all in cases 3 and 6 can probably be attributed to inadequate dosage of chorionic gonadotropin. These subjects received a total of 160,000 and 240,000 international units, respectively, which was significantly less than the 290,000 international units required for the development of gynecomastia in the left mammary gland of case 5.

In three of the four subjects (cases 1, 2, and 4) tenderness on pressure over the areolar area preceded the appearance of a mass in the breast by approximately one week. Only one subject (case 4), however, complained of spontaneous pain. Once the gynecomastia developed, it progressed in size very slowly and ultimately became evident on gross inspection. However, from the very beginning a clearly defined, indurated, firm disc of glandular tissue could be palpated in the subareolar area. In no instance did the breast attain the size seen in the young female at puberty, but in each there was a soft dome- or cone-shaped swelling approximately twice the size of the underlying disc of glandular tissue. (It should be noted that the size of the gynecomastia indicated in Table 2 refers to this disc of tissue.) Except for tenderness, the nipples and areolae exhibited no changes, and none of the subjects developed any secretion.

A biopsy specimen was obtained from the involved breast in three of the subjects (cases 1, 2, and 5). The histological features were essentially the same in all (Fig. 1) and closely resembled those seen in cases of chorioepithelioma,[1, 2, 14, 15, 18, 19, 21] except for the absence of alveolar development and secretion. In each instance, the mass was composed of areolar tissue and fat with a few strands of hyalinized connective tissue separating islands of actively proliferating, thickened, tortuous, and branching ducts. Immedi-

ately surrounding each of the ducts was a mantle of loose, highly cellular connective tissue which had a laminated appearance in some areas.

Only in case 5 was it possible to follow the course of the gynecomastia after gonadotropin therapy had been discontinued. The glands remained enlarged for a period of two months and then returned to normal. However, even six months later a small fibrotic nodule could be palpated beneath the areola on both sides.

No detectable changes occurred in the breasts of the female cirrhotic control who received 60,000 international units of chorionic gonadotropin. Conceivably larger doses might have produced hyperplasia, as in Kyle and O'Donovan's case.[65] Of interest in this connection are reports of mammary hyperplasia in women with chorioepitheliomas.[64, 65]

Testes. Biopsy specimens obtained before gonadotropin therapy revealed varying degrees of atrophy of the seminiferous tubules and arrest of spermatogenesis in every case (Table 3). This was most marked in case 5, the patient with pituitary deficiency, who also showed considerable thickening of the basement membrane and peritubular fibrosis (Fig. 2A). The basement membrane of the tubules was likewise thickened in the cirrhotics, but to a lesser degree, (Fig. 3A), and there was slight peritubular fibrosis in the "normal" control (Fig. 4A). As already mentioned, no testicular tissue was obtained in case 4, and only sections of the epididymal duct showing varying degrees of epithelial atrophy and degeneration were demonstrated (Fig. 5A).

The Leydig cells appeared to be normal in number, or only slightly decreased, in all but the subject with pituitary deficiency, in whom only rare Leydig cells could be found (Fig. 2A). In contrast, the number of Sertoli cells was slightly diminished in all cases.

Judging from the findings on subsequent biopsy, the administration of chorionic gonadotropin produced the following effects on the testis: (a) more active spermatogenesis in the cirrhotic (case 1), without any detectable increase in Leydig or Sertoli cells (Fig. 3B); (b) no significant change in any of the elements in the patient with pituitary deficiency (Fig. 2B), and (c) a marked increase in the number of Leydig cells in the "normal" control (Fig. 4B). Chorionic gonadotropin also appeared to restore the tubular epithelium of the epididymal duct to a normal state in the patient with congenital (?) atrophy or aplasia of the testes (Fig. 5B).

Androgen excretion. As might be expected from previous reports,[66-68] androgen excretion, judged by the results of both the 17-ketosteroid deter-

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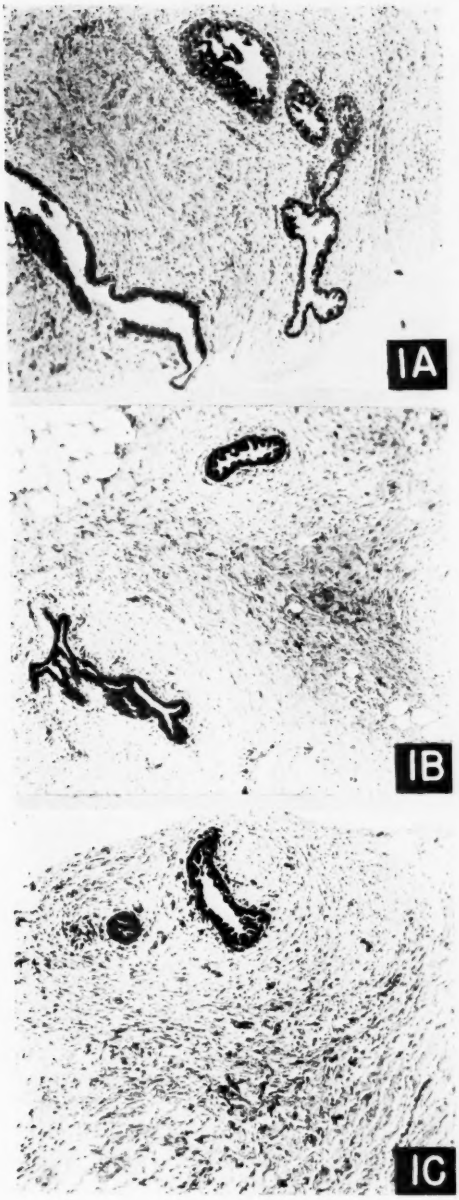
FIG. 1. Biopsies of hypertrophied mammary glands following chorionic gonadotropin administration.
A. Case 1 (x 75)
B. Case 2 (x 75)
C. Case 5 (x 75)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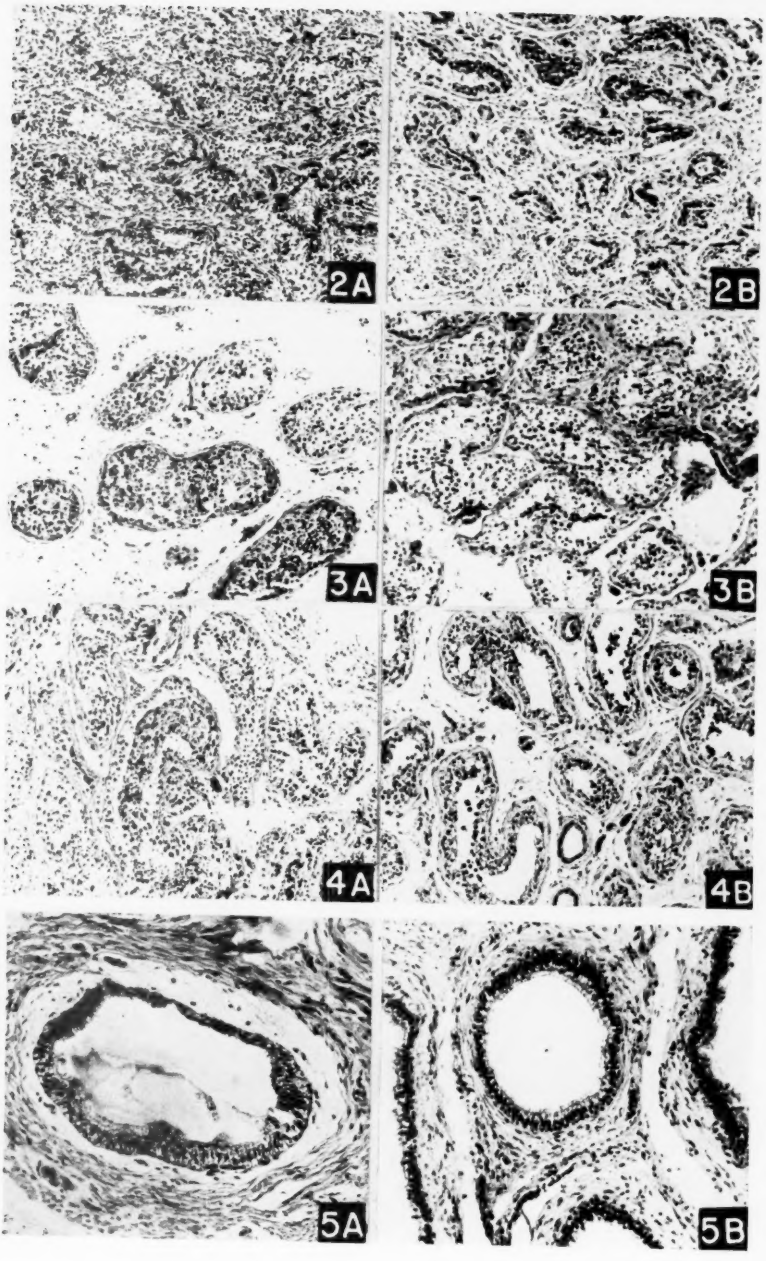
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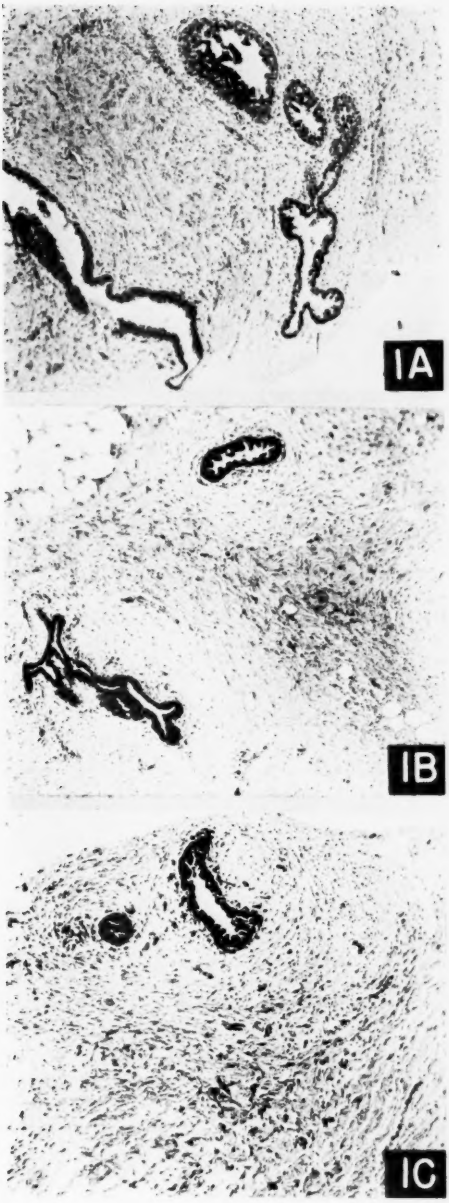
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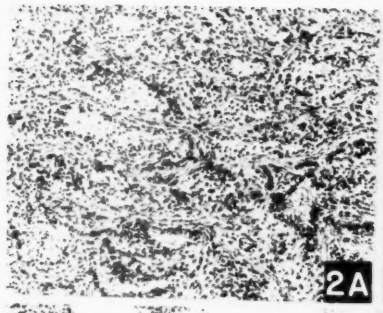
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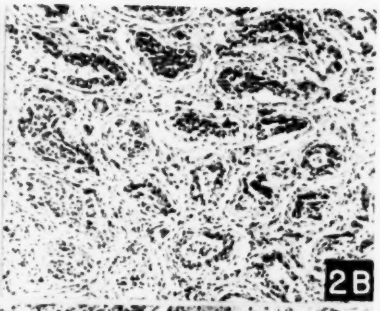
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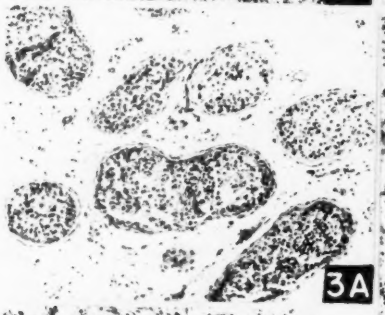
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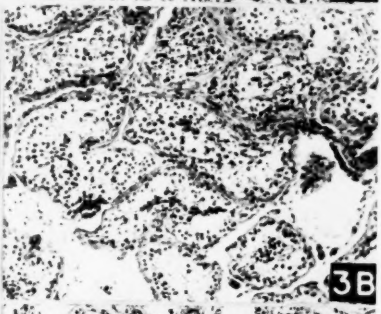
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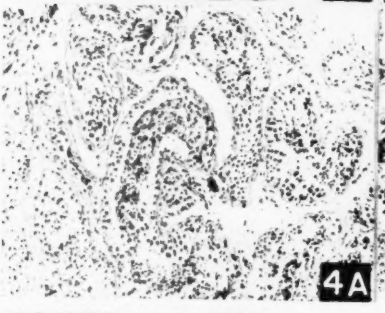
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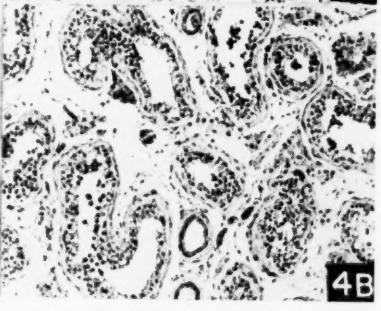
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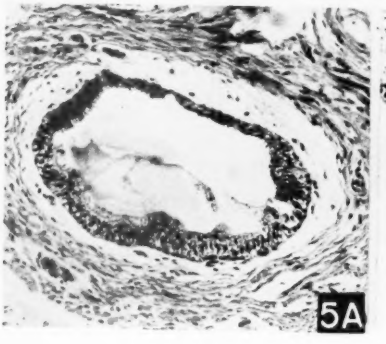
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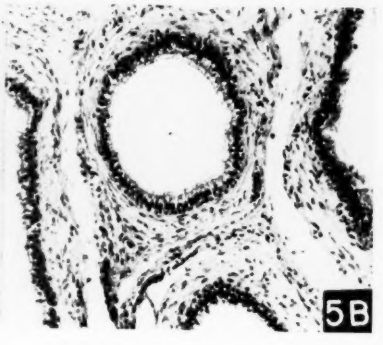
5B

minations and bioassays, was significantly decreased in all the cirrhotics, including the female, the patients with congenital (?) testicular atrophy, and the patient with pituitary deficiency (Table 4). This did not appear to be correlated with a decrease in Leydig cells, except in the subjects with pituitary deficiency and congenital aplasia of the testis. However, estimates of the number of Leydig cells based on histological examination of biopsy material do not take into account the volume of the testis, so that they may not be a reliable index of the amount of functioning interstitial tissue present.

Serial 17-ketosteroid determinations during the course of gonadotropin therapy (Table 4) revealed that, despite marked daily variations, there was a suggestive upward trend in excretion in two of the three male cirrhotics (cases 1 and 2) and in the female cirrhotic control (case 7). On the other hand, neither patient with congenital (?) testicular atrophy (cases 3 and 4) showed an increase in 17-ketosteroids.

Too few serial bioassays of androgen were carried out to permit any conclusions, but it is of interest that, whereas in case 1 the 17-ketosteroids rose from an initial level of 5.1 to a peak of 12.6 mg. per day after 82,000 international units of chorionic gonadotropin, androgen excretion remained at the low pretreatment level of 0.5 mg. per day. Similarly, in case 2 there was only a slight rise in androgen excretion, from 0.18 to 0.9 mg. per day, while the 17-ketosteroids increased from 4.4 to 9.5 mg. per day.

Of special interest was the occurrence of symptoms suggesting androgen stimulation in three of the subjects receiving gonadotropin. In one of the cirrhotics (case 2) frequent erections were first noted on the eighth day of therapy, even before there had been any significant increase in 17-ketosteroid excretion. Similarly, in case 4, the patient with congenital (?) atrophy or aplasia of the testes, there was a marked increase in libido and potency in the ninth week of treatment, and, in addition, an unmistakable increase in the beard necessitating more frequent shaving. In case 5, the

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FIG. 2. Testicular biopsies before and after chorionic gonadotropin administration in a subject with pituitary deficiency (case 5).
A. before therapy (x 130)
B. after 510,000 international units (x 130)

FIG. 3. Testicular biopsies before and after chorionic gonadotropin administration in a subject with Laennec's cirrhosis (case 1).
A. before therapy (x 130)
B. after 68,000 international units (x 130)

FIG. 4. Testicular biopsies before and after chorionic gonadotropin administration in a "normal control" (case 6).
A. before therapy (x 130)
B. after 240,000 international units (x 130)

FIG. 5. Biopsy of scrotal contents before and after chorionic gonadotropin administration in a subject with congenital (?) aplasia or atrophy of the testes (case 4).
A. before therapy (x 180)
B. after 195,000 international units (x 180)

TABLE 3

TESTICULAR BIOPSIES BEFORE AND AFTER CHORIONIC GONADOTROPIN ADMINISTRATION

Case		*Seminiferous tubules*						*Interstitial tissue*	
		Spermatogonia	*Spermatocytes*	*Spermatids*	*Spermatozoa*	*Basement membrane*	*Sertoli cells*	*Leydig cells*	*Fibrosis*
1.	before	++	++	+	0	++	++	+++	0
	after	++	++	++	+	++	++	+	0
2.	before	++	++	+	0	++	++	+++	0
3.	after	+	0	0	0	++	++	++	0
4.	before	no testicular tissue found; epididymal duct epithelium atrophic and shows degenerative changes.							
	after	no testicular tissue found; epididymal duct epithelium normal.							
5.	before	++	+	0	0	++	++	0	++
	after	++	+	0	0	++	++	0	++
6.	before	++	++	+	0	++	++	++	+
	after	++	++	+	0	++	++	++++	0

Cells: normal in number +++
slightly to moderately subnormal in number ++
markedly subnormal in number +
absent 0
increased in number ++++

Basement membrane: normal +
slightly to moderately thickened ++
markedly thickened +++

Interstitial fibrosis: absent 0
slight to moderate +
marked ++

The authors are indebted to Drs. John Berg and Averill Liebow of the Department of Pathology, Yale University School of Medicine, for examining these biopsy specimens and tabulating the data in this chart.

patient with pituitary deficiency, an increased growth of beard was noted at two weeks, and frequent erections at three weeks.

Estrogen excretion. Bioassay of the urine revealed that two of the male cirrhotics (cases 1 and 2) were excreting slightly more estrogen than normal, and during the administration of chorionic gonadotropin the amount increased significantly (Table 4). Chemically determined "estroids," on the other hand, remained within the normal range, although in case 2 there was a similar tendency to rise during gonadotropin therapy.

Case 3, the cirrhotic with congenital (?) testicular atrophy, had an abnormally high "estroid" excretion. During gonadotropin therapy the level fell transiently and then returned to the initial level. The fall in estrogen excretion could not be correlated with any change in 17-ketosteroids (Table 4).

Estrogen excretion was within normal limits in case 4, the patient with congenital atrophy or aplasia of the testes, but was abnormally low in case 5, the patient with pituitary deficiency (Table 4). Unfortunately, it was not possible to carry out serial estrogen assays during gonadotropin therapy in these cases.

The female cirrhotic had an abnormally low level of estrogen excretion, but this rose to within the normal range during chorionic gonadotropin administration (Table 4).

Gonadotropin excretion. Both subjects with congenital (?) testicular atrophy (cases 3 and 4) and the female cirrhotic (case 7) excreted abnormally large amounts of FSH, no doubt reflecting a "castration effect" on the pituitary (Table 4). On the other hand, as might be expected, the patient with pituitary deficiency (case 5) excreted very little. In the two cases of uncomplicated cirrhosis, FSH excretion was low in one (case 1) and low normal in the other (case 2). Concomitant with the increase in estrogen excretion noted during gonadotropin therapy in these two cases there was a fall in FSH excretion. This decrease was not noted during gonadotropin therapy in case 4, the patient with congenital (?) atrophy or aplasia of the testes; in fact, FSH excretion rose from 70 to > 160 m.u.u. per day. Unfortunately, parallel studies of estrogen excretion were not carried out in this case, so that it cannot be stated whether there was a reciprocal relationship between estrogen and FSH excretion. In view of the well-recognized fact that estrogen administration will reduce the increased gonadotropin excretion of eunuchoid men,[60] it is difficult to reconcile the high level of urinary gonadotropin in case 3 with the apparent increase in "estroid" excretion (Table 4), unless it is assumed that the nature of the compounds from which the latter was derived differed from most of the natural and synthetic estrogens.

Evidently none of the injected chorionic gonadotropin was excreted in the urine in cases 1 and 2 who received 2000 international units daily. This

TABLE 4

DAILY URINARY EXCRETION OF ANDROGEN, ESTROGEN, AND FOLLICLE-STIMULATING GONADOTROPIN

Case	*Day of gonadotropin administration*	*Cumulative dose of gonadotropin*	*Appearance of gynecomastia*	*Androgens*		*Estrogen*		
				17-ketosteroids	*Bioassay*	*"Estroids"*	*Bioassay*	*F.S.H.*
		units		*mg.*	*mg**	*mcg.†*	*mcg.*	*m.m.m.*
Normals								
Males				10–25	2–10	20–80	0.1–6	10–40
Females				6–20	1–10	40–120		10–50
1	—2	0		5.1	0.5	24	4	5
	—1	0		5.3		14		
Cirrhosis	1	2,000		4.1		39	3.6	
	6	12,000		6.2		14	.6	>20
	8	16,000		7.9		44		
	13	26,000		1.8		14		
	15	30,000		6.8				
	20	40,000		6.1		7	2.9	
	22	44,000		3.4		27		
	27	54,000		6.8	0.3			
	34	68,000	*	8.5				
	41	82,000		12.6	0.5			
	48	96,000		9.1		20		
	55	110,000		6.9		38	9.6	<5
	62	124,000		5.0	0.4	20	8.8	
2	—1	0		4.4	0.2	13	3.9	18
Cirrhosis	1	2,000		7.5			5.2	
	6	12,000		6.6		56		5
	8	16,000		7.2		21		
	13	26,000		8.4				
	15	30,000		7.2				
	20	40,000		4.9	0.7	48	7.2	
	22	44,000		8.3				
	27	54,000		7.4			16	
	29	58,000	*					
	34	68,000		7.1				
	41	82,000		9.5			10	
	48	96,000		6.0				
	55	110,000		7.1	0.6	70	11.2	<5
	62	124,000		7.7				
	76	152,000		9.5	0.9	29	9.6	
3	—3	0		5.9		130		160
Cirrhosis and	3	12,000		2.4		34		
Congenital (?)	6	24,000		3.7		77		
Testicular	10	40,000		3.6		110		
Atrophy	18	72,000		5.7				

Case	Day of gonadotropin administration	Cumulative dose of gonadotropin	Appearance of gynecomastia	Androgens		Estrogen		F.S.H.
				17-ketosteroids	Bioassay	"Estroids"	Bioassay	
		units		mg.	mg.*	mcg.†	mcg.	m.u.s.
4	—43	0		8.8	1.0		<3.0	70
Congenital (?)	—42	0		5.2				
Testicular	14	30,000		7.1				
Atrophy	53	115,000	*	5.1				>160
5	—19	0		3.5	0.4	0.8	<1.4	<5
Pituitary	0	0		5.3				
Deficiency	43	172,000	*					
7	—2	0						120
Female	0	0		2.3	0.1	21	<1.5	
Cirrhotic	1	2,000		1.0				
	6	12,000		2.7				
	7	14,000		3.3		74		
	11	22,000		3.1		16		
	12	24,000		2.7		51		
	14	28,000		6.0		85		
	17	34,000		5.7				
	23	46,000		11.3				
	24	48,000		3.4				

* androsterone equivalent (1 mg. = 10 international units of androgen).
† estrone equivalent (1 microgram = 10 international units of estrogen).

is in keeping with the observation of Lloyd and his associates[70] that it takes at least 5,000 international units in a single dose to produce an increase in urinary gonadotropin in non-pregnant females, and the report of Brown and Bradbury[71] that it requires daily injections of 10,000 to 20,000 international units to produce a positive Ascheim-Zondek reaction. It is highly probable, however, that the increase in urinary gonadotropin in case 4 was due to the excretion of chorionic gonadotropin, since in this instance the individual doses were 5,000 units administered thrice weekly.

Comment

The amount of chorionic gonadotropin injected in these experiments was well within the range secreted by the tumor in cases of chorioepithelioma with spontaneous gynecomastia, and was probably less than average considering the large amounts usually excreted in the urine.[5] For example, in Hamburger's case[17] the daily urinary excretion of chorionic gonadotropin was 600,000 international units, and even in a case like Laipply and Shipley's[19] in which urine contained only 300 mouse units, the secretion of

the tumor must have been very much greater considering the fact that only a fraction of injected chorionic gonadotropin is excreted in the urine.[70, 72, 78] Similarly, the duration of chorionic gonadotropin administration in these experiments was well within the range of time it usually takes for gynecomastia to develop in patients with chorioepithelioma. Obviously, it is impossible to determine the age of a tumor precisely, but in the 46 cases reviewed by Gilbert[5] there were 25 in which the date of onset of both testicular tumor and gynecomastia was given. In these, gynecomastia appeared on an average of 5.8 months after the presence of the tumor was recognized, and in 14 of the 25 it appeared in 3 months or less.

If the assumption is correct that the chorionic gonadotropin used in these experiments was not contaminated significantly by the other hormones found in human pregnancy urine, it is reasonable to conclude that the spontaneous gynecomastia of chorioepithelioma, like the experimental variety described here, is due to chorionic gonadotropin, and not to the other hormones known to be secreted by chorionic tissue.

Of the possible contaminants found in commercial preparations of chorionic gonadotropin derived from human pregnancy urine, only estrogen, FSH, and prolactin need be considered in connection with possible direct or indirect mammogenic effects. Estrogen can probably be excluded, since it is eliminated by the Collip method of extraction. Traces of FSH and prolactin are known to be present in the preparation used, but it is stated that the amounts are too small to exert any clinical effect.[38] The relative freedom of "A.P.L." from contaminating FSH has been confirmed by others.[74] In the present experiments only the slight increase in spermatogenesis observed in case 1 suggested an FSH effect. However, this could have been due to an increase in androgen, which, under some circumstances, is likewise known to promote spermatogenesis,[75] or to the secretion of FSH by the pituitary under the influence of chorionic gonadotropin, as suggested by Evans and Simpson.[76]

There is evidence that, quite apart from its ability to stimulate lactation in the properly prepared gland, prolactin may also induce growth of the mammary gland.[77] However, Folley and Malpress,[78] who have reviewed this subject suggest that such growth is probably due to the presence in many prolactin preparations of contaminating adrenocorticotropin. It is unlikely, therefore, that prolactin contamination of the chorionic gonadotropin used in these experiments was responsible for the gynecomastia which was produced.

There is no evidence that chorionic gonadotropin is capable of eliciting a growth response in the mammary gland directly, although this possibility has not been completely excluded. It is more likely, however, that its action is mediated through the gonads, the pituitary, or the adrenals.

The evidence presented by Geschickter, Lewis, and Hartman[38] would appear to implicate the testis in the mammogenic action of chorionic

gonadotropin. These investigators found that chorionic gonadotropin induced mammary growth in the male monkey only when the testes were present, and concluded that this effect was mediated through an increase in estrogen production related to hyperplasia of the interstitial cells of the testes. This conclusion, based on the assumption that the interstitial cells are a source of estrogen and that growth of the mammary duct system is necessarily due to estrogen, is no longer tenable in the light of more recent evidence indicating that the Sertoli cells are the sole source of estrogen in the testis[34, 79] and that androgen is also capable of provoking mammary hyperplasia[80, 81] and gynecomastia.[82] It must be assumed, therefore, that, in the monkey at least, the mammogenic effects of chorionic gonadotropin are mediated either through the interstitial cells by an increase in androgen secretion, or through the Sertoli cells by an increase in estrogen. However, it does not necessarily follow that the same is true in all species. Thus, for example, Lewis, Turner, and Gomez[83] were unable to produce growth of the mammary glands in intact male mice with large doses of chorionic gonadotropin.

What rôle the testes played in the present experiments is still uncertain but the occurrence of gynecomastia in the virtual absence of the testes in case 4, and the failure to demonstrate interstitial- or Sertoli-cell hyperplasia in the other subjects who developed gynecomastia strongly suggest that the mammogenic effects of chorionic gonadotropin were not mediated through the testes. The rise in 17-ketosteroids, on the other hand, which was observed in at least two of these subjects (cases 1 and 2) might be construed as evidence of testicular stimulation. However, an adrenal origin for these steroids cannot be excluded, especially since there was no concomitant increase in bioassayable androgen, and since there was an equivalent increase in 17-ketosteroid excretion in the female control. The observations of Reifenstein and his associates[84] may be pertinent in this connection. These investigators described a 27-year-old man with congenital absence of functioning testicular tissue whose 17-ketosteroid excretion was high. This increased transiently, following the administration of chorionic gonadotropin and was interpreted as evidence of compensatory adrenal cortical hyperplasia. Similarly, the maintenance of secondary sex characteristics[84] and the delayed rise in 17-ketosteroid excretion following castration[85] have been ascribed to an increase in adrenal cortical activity.

The evidence presented strongly suggests that the mammogenic effects of chorionic gonadotropin observed in the present experiments were not mediated through the testes, but it cannot be regarded as conclusive, since it was not possible to preclude with certainty the presence of testicular remnants in case 4.

The frequency with which hyperplasia of the pituitary is found in cases of testicular chorioepithelioma[2, 3, 5, 7, 8, 10–13, 18, 23, 24] the development of gynecomastia in some patients with pituitary tumors,[86, 87] and the evidence compiled

by Turner and his associates[43–45] implicating the pituitary as the major pathway for stimuli to mammary growth, point to the pituitary as a possible site of chorionic gonadotropin action in the production of gynecomastia. However, it is difficult to reconcile the development of gynecomastia in the subject with pituitary deficiency (case 5) with this concept, although it must be conceded that some pituitary tissue probably remained and could conceivably have been stimulated. More impressive is the experimental evidence that the pituitary has no direct mammogenic action,[46–49] and that, indeed, in some species its presence is not essential for mammary growth.[48, 49] It is highly probable, therefore, that the mammogenic effects of chorionic gonadotropin are not mediated through the pituitary.

A more convincing case can be made for the participation of the adrenal cortex in the development of choriogenic gynecomastia. That the adrenal cortex may, under some conditions, play a rôle in mammary growth is indicated by the development of gynecomastia both in association with adrenal cortical tumors[50–52] and following the administration of adrenal cortical extract[54] and desoxycorticosterone acetate.[53] There is also evidence that, in the hypophysectomized squirrel at least, chorionic gonadotropin is capable of stimulating the adrenal cortex,[40] and the enlargement of the adrenals noted in cases of testicular tumor associated with a high gonadotropin output[24] is suggestive of a similar effect in man. The secretion of colostrum[2, 3, 5] and the development of alveoli[5, 10, 12] in cases of choriogenic gynecomastia point to a progesterone effect, which is confirmed by the presence of pregnandiol in the urine.[18, 19, 27] The adrenal cortex is a likely source of this material in the male but the presence of progesterone in placental tissue[28] suggests the chorioepithelioma itself as an alternative source. Support for the latter possibility is perhaps to be found in the observation that none of the experimental subjects who received chorionic gonadotropin alone developed mammary alveoli or secretion.

While the hypothesis that the mammogenic effects of chorionic gonadotropin may be mediated through the adrenal cortex is an attractive one, it must be pointed out that chorionic gonadotropin does not stimulate the adrenal cortex in all species,[37] nor do all cases of choriogenic gynecomastia show adrenal hyperplasia.[1, 2, 4, 9, 11, 14, 15, 21, 22] The effects of chorionic gonadotropin on the adrenal cortex in man have not been studied very extensively. Kyle and O'Donovan[33] administrated 62,000 international units over a period of 26 days to a young woman with pituitary hypogonadism and "adrenal androgen deficiency," and observed a slight increase in 17-ketosteroid excretion, which was accompanied by nodular hyperplasia of the mammary glands and cornification of the vaginal mucosa. The latter were interpreted as estrogenic effects indicative of ovarian stimulation, and, for that reason, it was concluded that the adrenal had not been stimulated and that the increase in 17-ketosteroids was of ovarian origin. The authors recognized that inconsistency of an increase in follicular activity following

the administration of a luteinizing gonadotropin thought to be virtually free of FSH, and suggested several possible explanations. However, they did not consider the possibility that the estrogenic effects might be of adrenal origin. Certainly this could not be excluded since the adrenal cortex is known to contain estrogenic compounds[85] and to be capable of secreting them under some conditions.[86]

Recently Landau and his associates[87] studied the metabolic effects of chorionic gonadotropin in normal and eunuchoid men, and presented evidence to show that these effects were mediated through the testes. However, they recognized that minimal adrenal influences could not be excluded. Moreover, the possibility that these might be enhanced by more prolonged and intense gonadotropin stimulation must be considered, since these authors used relatively small doses, 14,000 international units in six days, an amount far less than that used in the present experiments or excreted in the average case of chorioepithelioma.

Tentatively, the adrenal cortex appears to be the most likely site of action of chorionic gonadotropin in producing gynecomastia. However, more direct proof of this hypothesis is still needed. A study of the effects of large doses of chorionic gonadotropin in castrates and in adrenalectomized males might serve to exclude the testis and establish the adrenal cortex as the pathway through which chorionic gonadotropin exerts its mammogenic effect. Unfortunately, it has not been possible for us to carry out such studies because of lack of suitable experimental subjects, but it is hoped that others who have access to such subjects may be able to do so.

Summary

Large doses of chorionic gonadotropin, derived from human pregnancy urine and given over a relatively long period, produced gynecomastia in two cirrhotics, in a subject with congenital atrophy or aplasia of the testes, and in a patient with pituitary deficiency. In the two subjects studied serially there was a concomitant increase in 17-ketosteroid and estrogen excretion, which was also observed in a female cirrhotic control. However, there was little or no increase in androgen excretion by bioassay, and in none of the subjects with gynecomastia was there histological evidence of interstitial- or Sertoli-cell hyperplasia in the testis.

On the basis of these observations and a review of the pertinent literature the following tentative conclusions have been drawn:

(a) The gynecomastia seen in association with chorioepitheliomas is probably related to the secretion of chorionic gonadotropin and not to any of the other hormones known to occur in such tissue.

(b) The colostrum-like secretion and the mammary alveolar development seen in some cases are probably due to progesterone, derived either from the tumor itself or from the adrenal cortex.

(c) The effect of chorionic gonadotropin on the male mammary gland is not a direct one, but is probably mediated through the pituitary, testes, or adrenal.

(d) The adrenal cortex is a likely site for this action, but further studies are needed to exclude other possibilities.

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RELATIVE POLYCYTHEMIA — THE POLYCYTHEMIA OF STRESS*

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In a group of normal subjects going from sea level to approximately 16,000 feet we observed that very soon after arrival at high altitude there was hemoconcentration, as indicated by an increased red cell count and hematocrit, but a normal total red cell volume and a low plasma volume. This "apparent increase" in the number of red blood cells in the early days after arrival at high altitude is, of course, replaced by a real increase in red blood cells or an altitude polycythemia after the subject remains at altitude for longer periods of time, since altitude anoxia stimulates red cell production.[6]

At sea level we have observed patients referred to us with a probable diagnosis of polycythemia vera who have the same picture with reference to the red cell count and blood volume. These individuals have increased red-cell counts and hematocrits, but normal total red cell volumes and decreased plasma volumes.[2] Although nearly all of these subjects have lived and are living at sea level or near sea level, it is our belief that they exhibit a relative polycythemia due to hemoconcentration possibly brought about by various forms of stress other than anoxic stress. In a series of 215 patients with elevated red cell counts, approximately 175 had polycythemia vera where there was a real increase in total red cell volume.[5] Twenty-two had polycythemia with increased total red cell volumes secondary to pulmonary or cardiac disease, and the remaining 18 (the subject of this paper) were patients exhibiting an increased red cell count and hematocrit but having a normal total red cell volume and a decreased plasma volume.

In the following discussion of these patients we refer chiefly to the 17 out of the total 18 who had no other associated organic pathology. In one female patient (#15) the finding of relative polycythemia was associated with multiple myeloma.

Age and sex. This condition is predominantly found in the male. Of the 17 patients with no apparent organic disease, 15 were males (#3 and #17 were female) varying in age from 16 to 68 and averaging 43 years. This is in sharp contrast to polycythemia vera where the average age of onset

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was 52 years. The sex incidence is also in marked contrast to polycythemia vera where 57% were males and 43% were females.[5]

History. There is no typical history. Six patients complained of dizziness. About one half of the patients were thought to have some significant anxiety state or to be mildly psychoneurotic. Two patients (#2 and #12) had been placed on thyroid extract because of a low BMR and one patient (#3) had a history of hyperthyroidism.

Physical examination. Eleven of the 18 patients had the ruddy cyanosis characteristic of polycythemia vera. The heart was not enlarged on physical or x-ray examination. The liver was not palpable (13 patients) or was questionably felt on deep inspiration (4 patients). None of the patients exhibited palpable enlargement of the spleen.

Eight of the 18 patients were thought to be overweight. Nine were hypertensive with a blood pressure greater than 150 systolic or 90 diastolic.

Laboratory Studies

Methods. In addition to the usual history and physical examination, the following studies were carried out:

(1) Complete blood count

(2) Blood volume with P^{32} labeled red blood cells[2]

(3) In most of the patients the following additional studies were done:
   (a) urinalysis
   (b) total protein, A/G ratio, blood uric acid and total bilirubin
   (c) blood oxygen saturation by the Scholander-Roughton method[9]
   (d) Fe^{59} turnover studies by the method of Huff *et al.*[4]

Laboratory Data

Red blood count. Thirteen of the 17 patients (excluding #15—multiple myeloma) had initial red cell counts in excess of 5.5 million and ranging up to 7.35. The remaining 5 patients had red cell counts between 4.9 and 5.5 million* (Table 1). Thus there was no tendency toward the extremely high red cell counts seen in polycythemia.

Hemoglobin. The hemoglobin varied from 13.1 gm./100 ml. in a patient who had been previously treated by phlebotomy to 18.5 gm./100 ml.—again showing a distribution closer to the normal than is seen in polycythemia vera.

Hematocrit. Six patients had a hematocrit below 50; in the remaining 12 it ranged from 50 to 58.

White cell count. Seven patients had white cell counts above 10,000 but of these only two were above 11,000. (This does not include the patient with multiple myeloma.)

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* Higher counts were found by the referring physicians.

Reticulocyte count. The reticulocyte count varied from 0.2 to 1.4%.

Differential white cell count. No abnormal white blood cells were noted in the peripheral blood. This is unlike the situation observed in patients with polycythemia vera.

Bone marrow. Satisfactory bone marrow punctures were obtained in 13 patients, including the one patient with multiple myeloma. Excluding the latter patient, the differential counts showed an average of 27.9% nucleated red cells and an average total count of 182,000 red cells. In normal subjects the percentage of nucleated red cells equals 16%, and in polycythemia vera, 38%.[10] In only one case in the present series was this figure above 33%, and in that individual it was 52.5%. For the other 11 subjects the mean nucleated erythrocyte percentage was 24.7. Thus, there is no significant increase in the number of nucleated red cells in the bone marrow of this group. Although there is on the average a significant increase in the nucleated red cells in the marrow of patients with both primary and secondary polycythemia,[7, 10] study of the bone marrow puncture is not diagnostic.

Urinalysis. In three patients (#1, 2, 5) the urine contained occasional white cells, in one (#14) it contained in addition a slight trace of sugar and occasional granular and cylindrical casts.

Blood chemistry. In 13 patients the total proteins varied from 6.8 to 8.6 gm./100 ml. and averaged 8.0 gm./100 ml. The albumin varied from 3.8 to 6.9 gm./100 ml. and averaged 4.6. The globulin ranged from 1.5 to 4.8, averaging 3.5 gm./100 ml. The A/G ratio varied from 0.67 to 4.5 and averaged 1.5.

Bilirubin. In 7 patients the total serum bilirubin varied from 0.03 to 1.0 mgm.% and averaged 0.4 mgm.%.

Blood oxygen saturation. In 8 patients the blood oxygen saturation averaged 92.5%, which is in the range for normal subjects and for those with polycythemia vera.[8]

Blood volume. The characteristic finding in these patients was a low plasma volume. The average was 25.6 and the range 20.4 to 30.9 ml./kg. body weight (the lower limit of normal being 32 ml./kg.). The total red cell volume averaged 28.3 ml./kg. (normal for male is 29.9 and for female 27.0 ml./kg.).[1]

Fe^{59} turnover studies. These studies showed that the rate of disappearance of radioiron from the plasma and the amount of iron incorporated into red cells per day were normal and in good agreement with the patients' requirements. The characteristically rapid disappearance of radioiron from the plasma and the relatively high rate of utilization of iron for red blood cell formation seen in patients with polycythemia vera or secondary polycythemia[4] were not observed in this group.

TABLE 1

Pt.	Age	Height	Weight	Obesity	Ruddy cyanosis	B.P.	RBC†	Hgb.	WBC	Platelet	ml./kg. Body weight				Hmt.	Date
											Blood volume	T.R.C.V.	Plasma volume			
1.	44	6′ ½″	96 kg.	+	+	130/85	6.54	18.1	14,900	270,000	51.8	28.5	23.3		55	3–3–49
2.	43	6′ 0″	81.4		—	130/78	6.04	15.0	10,900	260,000	54.4 (4432 cc.)	26.1 (2127 cc.)	28.3 (2305 cc.)		48	3–9–51
			84.5			120/75	5.16	14.0	12,300	290,000	56.9 (4804 cc.)	27.6 (2330 cc.)	29.3 (2474 cc.)		48.5	9–28–51
3.	68	5′ 2½″	56.7		+	260/140	6.82	17.4	10,200	380,000	45.4 (2578 cc.)	25.0 (1418 cc.)	20.4 (1160 cc.)		55	2–9–51
			53.4			210/135	6.02	16.7	9,300	320,000	52.7 (2812 cc.)	29.0 (1547 cc.)	23.7 (1265 cc.)		55	11–16–51
4.	39	6′ 4″	135.4	+	+	125/75	6.04	17.1	10,100	360,000	52.1	23.4	27.6		45	1–16–50
5.	39	5′ 10″	93.2	+	+	155/115	7.35	17.5	8,000	240,000	52.4	26.7	25.1		51	10–21–49
			91.0			126/88	5.46	16.9	8,400	360,000	49.0	29.4	19.6		60	10–15–51
6.	59	5′ 9″	74.1		+	142/82	5.76	14.6	7,800	440,000	61.4 (4548 cc.)	30.7 (2274 cc.)	30.7 (2274 cc.)		50	1–23–51
			74.5				4.76	14.8	8,050	320,000	60.8 (4532 cc.)	30.4 (2266 cc.)	30.4 (2266 cc.)		50	10–31–51
7.	26	6′ 0″	76.8		—	140/80	5.78	16.6	6,600	320,000	54.4	28.4	25.6		52	1–30–51
8.	41	5′ 7″	83.2	+	+	130/98	5.37	15.6	7,200	310,000	54.7	26.8	27.3		49	2–28–50
9.	16	5′ 6½″	65.5		—	110/62	5.68	16.8	7,300	390,000	55.8	27.0	28.5		49	3–8–50
10.	57	5′ 10¼″	99.5	+	+	120/80	5.24	17.8	7,800	260,000	58.6 (5837 cc.)	32.8 (3268 cc.)	25.2 (2509 cc.)		56	2–27–50
			95.5			135/80	7.16	18.1	8,300	220,000	60.5 (5782 cc.)	36.3 (3469 cc.)	24.2 (2313 cc.)		60	2–27–51
11.*	46	5′ 7″	81.8	+	+	124/98	4.96	14.2	15,500	150,000	57.7 (4716 cc.)	28.0 (2287 cc.)	29.7 (2429 cc.)		48.5	2–17–50
							5.5	17.0	9,400		(5050 cc.)	(2190 cc.)	(2860 cc.)		47.5	10–10–51
12.	32	5′ 10″	89.5		—	148/105	6.15	18.5	7,100	180,000	56.6 (5056 cc.)	30.6 (3035 cc.)	26.0 (2030 cc.)		54	5–19–49
			86.4				5.10	17.2	8,300	242,000	58.8 (5077 cc.)	33.2 (2869 cc.)	25.6 (2208 cc.)		56.5	10–20–51
13.	47	6′ 0″	103.6	+	—	130/100	6.61	14.8	4,400	320,000	53.1	26.5	26.5		50.0	5–15–50
							5.18	13.8	4,500	210,000						
14.	33	5′ 6″	64.5		—	122/68	5.32	17.4	10,850	460,000	63.5 (4098 cc.)	31.8 (2049 cc.)	30.5 (1967 cc.)		50	1–4–51
			63.6				5.86	16.7	10,200	304,000	71.2 (4527 cc.)	36.3 (2309 cc.)	34.9 (2218 cc.)		51	9–22–51
15.	65	4′ 8″	47.9		+	182/98	4.95	17.5	15,000	380,000	52.6	27.0	25.6		51	1–18–49
16.	18	.....	79.1		+	118/72	5.90	16.7	6,900	420,000	65.4	36.0	29.4		55	12–19–51
17.	46	.....	60.1			160/100	5.95	13.1	11,000	360,000	59.4	28.5	30.9		48	9–7–51
18.	62	5′ 1″	76.8	+	+	185/85	5.79	15.8	7,130	260,000	57.7	33.5	24.2		58	3–12–52

T.R.C.V. = Total red cell volume.
Hmt. = Hematocrit.
* Second blood volume done by Dr. J. Stanton, Evans Memorial Hosp., Boston, Mass.
† Although some of these red cell counts are not elevated, they had been previously, since all of these patients were referred to us with a diagnosis of polycythemia vera.

Follow-Up

Follow-up studies of the history and physical findings and particularly of the blood volume were obtained in 8 patients at intervals varying from 6 to 24 months. During this interval the patients did not receive specific therapy and were not phlebotomized. The pertinent physical and hematological findings are presented in Table 1. In general there was no striking change in the symptomatology, physical, or hematological findings. In most of the patients the peripheral blood counts were in good agreement, particularly the hemoglobin concentration. (Patient #11 was seen here after previous phlebotomies and in the interval probably repleted his iron stores sufficiently to account for the increased hemoglobin.) In no instance was a significant change in the blood volume noted.*

Discussion

The typical patient with this type of polycythemia is a male in the fourth or fifth decade who is overweight, has a ruddy cyanosis, and may complain of vertigo. There is a moderate elevation of the red count, hemoglobin, or hematocrit with a normal white count and platelet count. Aside from the obesity, ruddy cyanosis, and moderate hypertension in half the cases, the physical examination is noncontributory. There is a normal total red cell volume but an abnormally low plasma volume leading to an apparent elevation of the red cell count, hemoglobin, or hematocrit. Fe^{59} turnover data are not characteristic of polycythemia vera or of secondary polycythemia and show a rate of incorporation of iron in the red blood cells that is within normal limits. In many respects these patients seem to fit into the group of patients described by Gaisbock in which there were polycythemia and hypertension but no splenomegaly.[8] It should be noted that the mean white blood cell count of the 17 patients (excluding patient #15) was 9,060. This was not significantly different from the mean white cell count (9,160) observed in 200 pre-employment examinations in this laboratory and is in marked contrast to that observed in polycythemia vera[2, 3] indicating that, at least from this standpoint, the bone marrow is not abnormal.

One is tempted to relate the blood volume changes in these patients to some form of emotional stress and to class this condition as a psychosomatic phenomenon. Our observations in normal subjects undergoing altitude anoxia show a similar change in blood volume, and in these cases there is a measurable physiological stress.

Therapy directed toward decreasing the total red cell volume is contraindicated in these patients since they have a normal total red cell volume.

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* In these patients the blood volume is tabulated in both absolute values and in terms of ml./kg. body weight to permit comparison when there has been a significant weight change.

It is important that the true condition of these patients be recognized and treated accordingly. Unlike patients with polycythemia vera they should not receive P^{32} therapy.

Summary

The findings have been presented in 18 cases of relative polycythemia. There was an increase in the red cell count, hemoglobin, or hematocrit, but a normal total red cell volume, the increase being due to a pathologically low plasma volume. In 17 of these cases no associated disease could be found. Sixteen of the 18 patients were males. Eight were obese and nine were hypertensive. The psychiatric background of some of these patients indicated that they had been subjected to undue nervous stress and strain. Although it is known that anoxic stress produces hemoconcentration, the possible effect of nervous stress and strain on blood volume is reported here for the first time.

Case Reports

Patient 1: A 44-year-old white physician was first seen in February 1949 with a chief complaint of fatigue of ten years' duration. In 1947 he had thromboses in the legs and several pulmonary infarcts for which he received continuous dicoumarol therapy. In May 1948 he had massive pulmonary bleeding which necessitated tracheotomy and transfusion of 3500 ml. of blood. In January 1949 he developed dizziness, occipital headache, and precordial pain which lasted several days. At that time an elevated red cell count and hemoglobin were observed. Physical examination revealed an obese white male with slight reddish cyanosis of the ear lobes and nail beds. The only other positive physical finding was a nonpitting, firm edema of legs, ankles, and feet. He had a history of numerous domestic, social, and economic problems of a very trying nature and later was incarcerated in a Federal Penitentiary.

Patient 2: A 43-year-old white salesman had attacks of faintness and weakness and some shortness of breath following pneumonia in 1938. These varied in frequency from one to four times per month. He had frequent frontal headaches lasting from one hour to one day not relieved by aspirin but alleviated by nosedrops. Because his basal metabolic rate was —35 he was given two grains of thyroid daily. On physical examination there was noted some obstruction of the nasal air passages; the thyroid was palpably enlarged; the liver was palpable at the costal margin. BMR was —3 and —5. Blood cholesterol was 155 mgm.%, total; 34 mgm.%, free cholesterol. Blood protein-bound iodine was 5.9 μg.%. At the time the patient was seen in this laboratory a diagnosis of hypothyroidism could not be established.

Patient 3: A 68-year-old white female presented herself with a one-year history of dizzy spells occurring every two months and lasting two to three days. She was a known hypertensive of seven years' duration. Three years ago she had a carcinoma of the bridge of her nose treated successfully with x-ray. She had had hypertension for the past seven years. Fifteen years ago a diagnosis of hyperthyroidism was made and she was treated for one year with iodine. Twenty-two years ago she had a radical mastectomy for carcinoma. There have been no recurrences of these past ailments. On physical examination a questionable slight exophthalmos and a definite lid lag were noted. The pulse was 102 and there was a Grade II systolic murmur at the aortic and mitral areas. The blood pressure was 260/140. She was very restless and fidgety.

Patient 4: A 39-year-old white male was struck on the head with a hammer 22 months before being seen in this laboratory. This was followed by a nearly continuous throbbing left parietal headache which has caused him to be irritable and "nervous." Six months ago he had a copious epistaxis with relief of the headache for one week. The only positive significant physical finding other than those detailed in the table was hyperesthesia of the scalp in the left parietal area.

Patient 5: A 39-year-old white male complained of low back pain of one week's duration, an erythematous rash of many years' duration, and a history of pyelitis 15 years ago. On physical examination an erythematous rash was noted on the face, chest, and back.

Patient 6: A 59-year-old white male had three attacks of severe dizziness during the past ten years. For several years his red blood count was high and his hematocrit slightly above normal. On physical examination the positive findings were a ruddy complexion, and a slight hyperemia of the mucous membranes of the nose, mouth, and pharynx.

Patient 7: A 26-year-old white male gave a history of fatigue and occasional attacks of tachycardia for the past two years, following infectious mononucleosis. On physical examination there was congestion of the mucous membranes of the nose, and a faint systolic murmur was heard at the base of the heart.

Patient 8: A 41-year-old male, who lived at an altitude of 5,000 feet complained of intermittent right lower quadrant pain, not associated with meals, for three to four years. For the past month he noted redness and burning of the tongue.

Patient 9: A 16-year-old white male complained of "heartburn" and belching after meals. On physical examination hyperemia of the pharynx was noted.

Patient 10: A 57-year-old white male, who lived at an altitude of 6,700 feet, complained of ascites and edema of two years' duration. In 1942 redness of the face and elevated red blood count were found and he was treated intermittently with phenylhydrazine and venesections. On physical examination an area of dullness with inspiratory rales was noted in the right chest posteriorly. X-ray showed no pulmonary disease. A systolic murmur was heard at the apex. X-ray of the abdomen showed enlargement of the spleen although it was not palpable.

Patient 11: A 46-year-old white male clergyman had a coronary thrombosis in 1945 from which he recovered after a prolonged period of rest. Since that time he has had typical anginal pain with exertion. In 1947 he had an elevated red blood count and hemoglobin and was treated by phlebotomy. He had malaria in 1932. On physical examination the optic fundi showed slight engorgement and there was slight enlargement of the isthmus of the thyroid. The liver and spleen were not palpable.

Patient 12: A 34-year-old white male gave a history of occipital headache, vertigo, and epigastric distress of ten to thirty minutes' duration. In October 1948, because he had a low BMR, he was given ½ grain thyroid three times a day; this was increased to 1 grain three times a day in January 1949.

The past history includes a possible attack of malaria in 1946. Physical examination was entirely noncontributory. From the neuropsychiatric standpoint the patient was described as being a nervous, hard-working person with inward repressions.

Patient 13: A 47-year-old Negro physician developed a headache nine days prior to his arrival at this laboratory. About one or two hours later he became dizzy and stumbled while walking. The dizziness was not a true vertigo. There was also an associated vomiting. There was no loss of consciousness. He was seen by his own physician who made a tentative diagnosis of labyrinthitis and prescribed dramamine and ammonium chloride. The past history included malaria. On physical examination, moderate injection of the pharynx was the only positive finding.

Patient 14: A 33-year-old white male first noticed attacks of vertigo while serving with the Navy during the Second World War and while under fire in the jungle. These started with a dizzy sensation after which he became nauseated but did not vomit. On some occasions he fell. The attacks, generally lasting five to ten minutes, recurred every two to three months, but have been less severe during the past year. In 1943 his red count was found to be high. In 1942 he had malaria and in 1944 he was thought to have a palpable spleen. On physical examination the positive findings included slight injection of the sclerae, moderate engorgement of the fundi oculi, and injection of the mucous membranes of the pharynx.

Patient 15: This 65-year-old white female had multiple myeloma.

Patient 16: An 18-year-old white male was referred by his physician with a history that in September 1949 the joints of his hands were swollen and thought to be involved

by arthritis. He was treated with salicylates up to 40 grains a day with definite improvement. However, the joints in his hands still remained larger than was felt normal for his size. He also complained of occasional fainting spells. A blood count by the referring physician showed a hemoglobin of 19.5 gms. and a red count of 6.25 million. The white blood and differential count were normal. In September 1949 and in November 1949 he had an upper respiratory infection at which time again a slight elevation of the hemoglobin and red count was seen. This elevation persisted throughout January 1950 and until November 1951 when he was seen in this laboratory. He then denied any joint swelling as described by his referring physician, and also denied the fainting episodes. On examination he had moist hands with considerable diaphoresis. He appeared to be quite disturbed, afraid, and very nervous. No other positive physical findings were noted.

Patient 17: A 46-year-old white female gave a history of progressive fatigue and nervousness of two years' duration. In 1949 and 1950 she was given tetanus antitoxin and in 1951 tetanus toxoid. Both antitoxin injections were followed by serum reactions, and the toxoid by a sensation of retro-orbital pressure. At the present time she complains of occasional headaches, urinary frequency, tachycardia, and substernal distress. The only significant finding on physical examination was a pulse of 100 with blood pressure of 160/100. From the neuropsychiatric standpoint the patient exhibited considerable apprehension and it was thought that most of her symptoms were on a neuropsychiatric basis. This patient consulted a large number of physicians about her problem, even a previous husband who was a physician.

Patient 18: A 62-year-old real estate man was referred to us with a history of elevation of the red blood count for the past six years. Two years ago diagnosis of polycythemia was made. The history included two years of easy fatiguability, dizziness, loss of mental alertness, and headaches. On physical examination the only remarkable finding was the obesity (44% of his body weight was fat as calculated from measurement from the body water and body specific gravity), and moderate hypertension (blood pressure 185/85).

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THE TREATMENT OF HYPERTENSION: A FIFTEEN-YEAR FOLLOW-UP*

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In 1936 the late Dr. Francis G. Blake encouraged me to work with my surgical colleague, Dr. Ashley W. Oughterson, in the selection of patients for the surgical treatment of hypertension and in their subsequent postoperative course. The patients, twelve in number, were treated surgically by one of us (A.W.O.) by subdiaphragmatic splanchnic resection between 1937 and 1940 and were reported in detail in 1941.[20]

Of these original twelve patients (eight female and four male), the ages ranged from twenty-three to fifty-three years; eleven were white and one was colored. The blood pressure ranged from 250 to 300+ mm. Hg. systolic and 120 to 170 mm. Hg. diastolic pressure in the females with the exception of one, in whom the original pressure was 180/105. In the males the pressure ranged from 230 to 270 mm. Hg. systolic and 130 to 165 mm. Hg. diastolic pressure with the exception of one patient in whom the original blood pressure was 160/105. Of this entire group the hypertension had returned to its preoperative level in each instance within six to twelve months and now, twelve to fifteen years later, there are only three survivors, all females. Each of the survivors has severe hypertension despite the fact that one of them was operated upon again (lumbo-dorsal sympathectomy) five years ago.

Eight of these twelve patients were younger than forty years of age at the time of operation and three of the four males were under the age of forty. Two of the males died within eight and fourteen months, one three years and one four and a half years postoperatively. No patient in this group had any truly objective postoperative improvement. All of the four male patients were considered to have "malignant" hypertension, while four of the eight female patients were so designated.

Since 1940 it has been my privilege to follow closely an additional forty patients who were surgically treated by the more extensive thoraco-lumbar sympathetic resection of the Smithwick variety.[28] This group was operated upon in the period from 1940 to 1947 and is composed of twenty-six females and fourteen males, with ages varying between twenty-two and fifty-four years. Of this latter group there is only one survivor among the males and fifteen survivors among the females. Of this group, twelve of twenty-six

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FIGURE 1

SURVIVAL CHART
12-TO 15-YEAR FOLLOW-UP
SUBDIAPHRAGMATIC SYMPATHECTOMY

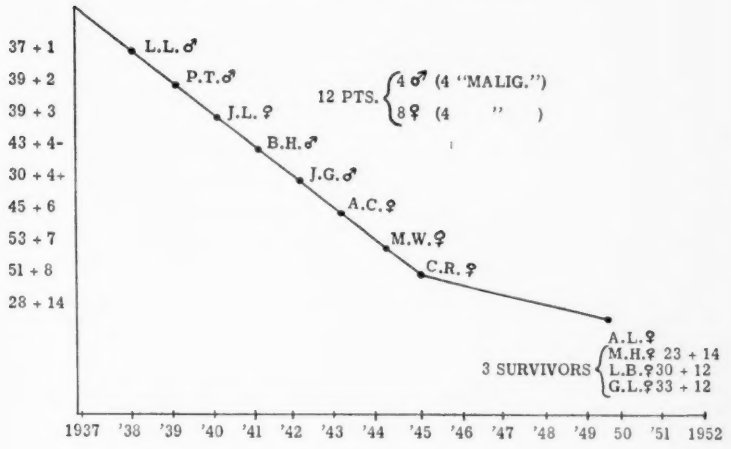
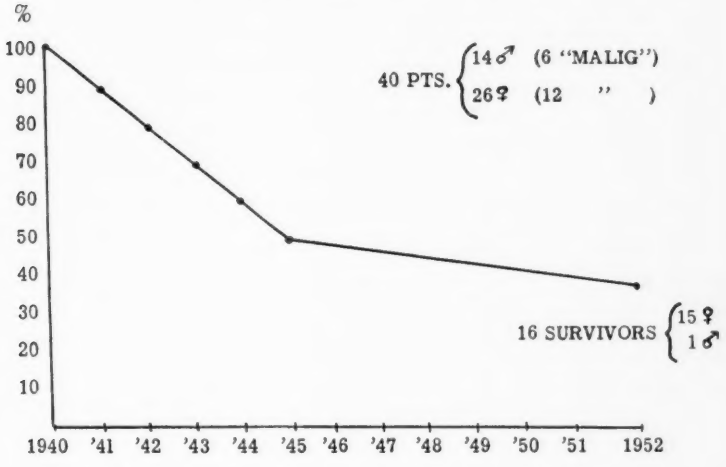


FIGURE 2

SURVIVAL CHART
5 TO 12-YEAR FOLLOW-UP
LUMBO-DORSAL SYMPATHECTOMY



females and six of fourteen males gave evidence of "malignant" hypertension when first seen. Of those with "malignant" hypertension there are three survivors of twelve patients among the females and only one of the six among the males. The blood pressure ranged from 240 to 300+ mm. Hg. systolic and 120 to 180 mm. Hg. diastolic pressure in the females and from 230 to 280 mm. Hg. systolic and from 130 to 170 mm. Hg. diastolic pressure in the males. In this entire group of forty patients the blood pressure has returned to preoperative, hypertensive levels within six to twenty-four months and there has been little, if any, objective evidence of improvement.

FIGURE 3

40 PATIENTS - AGE RANGE 22-54
LUMBO-DORSAL SYMPATHECTOMY

DECADE	20 - 29	30 - 39	40 - 49	50 +
NO. OF PTS.	♂ 3 (1) ♀ 3 (1)	8 (4) 12 (6)	3 (1) 9 (3)	- (2)

() DENOTE "MALIGNANT" HYPERTENSION

Operative Criteria

In our previous study[20] we stated: "The criteria for suitable patients for this operation (sympathectomy) are not well defined." This statement is still true for, despite many diagnostic and prognostic criteria that have come and gone, none has proven of any real value in selecting those patients who might be expected to do well postoperatively. In general, such patients probably should be limited to those no older than fifty or fifty-five years of age and preferably to the twenty- to forty-year age group. Renal function should be unimpaired and the patient, if in heart failure, should be brought into cardiac compensation.

The Hypertensive Survey

Since there is a small percentage of hypertensive patients who may be dramatically cured surgically, every group of hypertensives should be searched carefully for one of the following causes of curable hypertension:

(1) Brain tumor with "central" hypertension
(2) Coarctation of the aorta
(3) Pheochromocytoma of the adrenal medulla

(4) Adrenal cortical tumor

(5) Unilateral renal disorder, provided the remaining kidney is normal.

It should go without saying that for every patient with hypertension, whether or not surgical therapy is contemplated, the following information should be secured:

(1) A carefully taken medical history which should include:
   (a) Family history
   (b) Marital history
   (c) Occupational history
   (d) Socio-economic history
   (e) Emotional history, including habits relating to drugs, tobacco, alcohol, and recreation

(2) A complete physical examination with particular attention to:
   (a) The eyegrounds
   (b) The heart
   (c) The vascular system
   (d) The kidney regions
   (e) The blood pressure measurement early and late in the examination and under variable states of emotional tension and relaxation. It is well at all times during such an examination to de-emphasize to the patient the importance of the level of the blood pressure.

(3) An X-ray and laboratory examination which should include:
   (a) X-ray of the chest for cardiac size and shape and to rule out coarction of the aorta.
   (b) Electrocardiogram. This is particularly important if serial E.K.G.'s can be taken over a period of years. The ballistocardiogram may be added as a supplementary aid when indicated.
   (c) X-ray of the abdomen with particular attention to the kidney region (calculi, polycystic kidneys, etc.)
   (d) An intravenous pyelogram and occasionally one must resort to retrograde pyelography for optimum visualization of the urinary tract.
   (e) X-ray of the skull may prove helpful but is not routinely necessary.
   (f) Urinalysis with particular attention to albuminuria and to the urine sediment. Addis counts may be helpful as a guide

in renal disease activity if they are performed by the same individual or by a small group of careful microscopists.

(g) Renal function may be measured grossly by the specific gravity and P.S.P. dye excretion and more adequately by the urine concentration and dilution tests. An N.P.N. or B.U.N. determination should be made on the blood since occasionally one may find elevation in the absence of albuminuria or abnormalities of the urine sediment. Urea clearance and other more complicated tests of renal function should be reserved for those patients where such study is indicated.

(h) Urine culture. This should always be routine in the work-up of hypertensives since much hypertension, especially in young females, is based upon pyelonephritis and frequently may be cured with antibiotics or chemotherapy.

The X-ray and laboratory survey should attempt to rule out acute or chronic glomerular nephritis, chronic pyelonephritis with extensive and permanent renal damage, amyloid disease of the kidney, congenital polycystic kidneys, abberant renal blood vessels, bilateral hydronephrosis (with or without calculi) secondary to long-standing lower urinary tract obstruction, since these conditions, among others, may cause hypertension.

Symptoms

It is quite well recognized that there are likely to be no symptoms of hypertension until the complications of hypertension express themselves by way of abnormalities in the retinal and cerebral vessels, the coronary or the renal circulation. It generally is conceded that headache is not a common symptom of hypertension until after the patient has been told that he has high blood pressure. This also is often true of such symptoms as nervousness, fatiguability, dizziness, tightness or pain in the occipital and posterior cervical region, insomnia, palpitation, and even precordial pain. These are among the classical symptoms of anxiety which so often are iatrogenic in origin.

Therapy

The variety of surgical operations, including the ultraradical adrenalectomy, that have been designed to correct hypertension, and the multitude of drugs and medical measures that have been used attest to the fact that as yet we do not have the answer. Many of my readers have witnessed the waves of enthusiasm that followed the introduction of the vasodilators, the thiocyanates, the various low-calorie, low-salt, and low-protein diets, pyrogen therapy, the cyclic re-introduction of the veratrum alkaloids, and now,

more recently, the sympathetic ganglion-blocking agents such as tetraethylammonium chloride, penta- (C_5) and hexamethonium (C_6) bromide and iodide, the homologue of hexamethonium (M & B 1863) and other compounds such as hydrazinophthalazine (Apresoline C-5968) and the hydrogenated alkaloids of the ergotoxine group (Hydergine).

While there are many who still are enthusiastic about the therapeutic value of the veratrum compounds, this group of drugs is difficult to control in clinical therapy. The incidence of toxicity is high and the toxic and therapeutic doses are separated by only a narrow margin of safety. It is doubtful whether their use should be attempted by the inexperienced. It also does not seem that the course of an appreciable number of hypertensive patients has been altered by this form of therapy.[3, 10, 28, 30, 33, 34, 39, 43]

During the past two years there has been increasing enthusiasm regarding the use of several members of the methonium group of drugs with their ability to perform a chemical sympathectomy by means of their ganglionic blocking properties.[4, 6, 11, 12] Pentamethonium (C_5) and hexamethonium (C_6) have received the greatest amount of attention. However, because of the inconstancy of dosage and the irregularity of absorption and individual response, we feel that these drugs may prove disappointing in the therapy of the hypertensive patient.[22]

It should be emphasized that in the treatment of either essential or malignant hypertension many writers claim a very high incidence of symptomatic relief for their patients with the particular form of treatment that they recommend, frequently without relating this improvement to any objective decrease in blood pressure. As one reviews the literature on this subject it appears that almost any form of therapy produces an objective drop in blood pressure in approximately 25 per cent and a symptomatic improvement in 50 to 75 per cent of patients. This degree of subjective improvement is not rare even when a placebo is used!

In the present state of our lack of understanding of hypertension it might well be harmful to stress the value of any potentially dangerous form of therapy when subjective improvement so often occurs with a placebo administered with enthusiasm and confidence on the part of the physician.

I should like to offer the thought that hypertensive patients who survive for long periods of time are not necessarily alive *because of* the surgical or medical treatment of their hypertension but perhaps occasionally *in spite of* the therapy that they have received!

Order of Choice in Therapy of Hypertension

(Following careful investigation of the patient)

GROUP OF CHOICE

(1) Superficial psychotherapy of sympathetic understanding and "good listener" variety by the family physician or the internist, the psychiatrist

to be asked for assistance only in those few who do not respond well, or moderately well, to the above.

(2) Rest. Reasonable physical rest and removal from, or alleviation of, emotional stress wherever and whenever possible. Prolonged bed rest is usually impractical and economically impossible.

(3) Mild sedation, eg. phenobarbital, grs. ¼, b.i.d., t.i.d., or q.i.d., especially during periods of emotional stress.

(4) Weight reduction for the obese.

(5) Low sodium diet for those who have cardiac or renal involvement. Beware of low sodium diet in excessively hot weather, in vomiting, diarrhea, or in renal "salt wasters."

Group of Less Value

(1) Veratrum alkaloids. These drugs are quite toxic and difficult to control.

(2) Methonium compounds. Their action is irregular and reactions are dangerous.

(3) Pyrogen therapy. This treatment is usually impractical because a long period of hospitalization is necessary.

Group of Least Value

(1) Thoraco-lumbar sympathectomy. This should be reserved for those unusual patients who, despite other more reasonable forms of therapy, continue a downhill course in "malignant" hypertension. This should constitute a very small percentage of the entire group.

(2) Adrenalectomy. Impractical and impossible beyond constant hospital or research control.

Discussion

Cardiovascular disease ranks number one as the arch enemy and killer of the human race. Hypertension, sometimes called a disorder of "civilization," is commonly associated with cardiac disease, renal disease, and arteriosclerosis but frequently is present, as essential hypertension, in their absence.

Since the maintenance of normal arterial pressure is regulated by a highly intricate and carefully integrated mechanism that is probably controlled through the central nervous system, it is not too much to expect that elevated arterial pressure may be brought about by interference with the regulatory mechanism at the level of the central nervous system, the autonomic nervous system, the vascular system, or the endocrine system by way of the hormonal and chemical imbalance contributed to by the pituitary gland and the cortex and medulla of the adrenal gland. This regulatory mechanism may be influenced also by the kidney. A patient with hyper-

tension, therefore, may appear to have his elevated blood pressure on an emotional, neurogenic, endocrine, humoral, chemical, metabolic, cardiac, vascular or renal basis, or on the basis of any combination of these.

It is now thought by many that the stimuli that may upset the blood pressure regulatory mechanism may come from the patient's external environment in the form of emotional trauma. This succession of emotional traumata may cause temporary or sustained elevation of pressure and eventually may affect the patient's entire internal environment. Since our physical and emotional make-up is so highly dependent upon the combination of the impact of our heredity and our environment, it is little wonder that the cause of hypertension in any one patient may be single or multiple and that hypertension may result from a single event or from a varied succession of events. These tend at first intermittently and later continuously to raise arterial pressure. Eventually perhaps as part of a process of adaptation, the central integrating mechanism controlling the basal arterial pressure may become set at a higher level, thereby establishing a vicious cycle of pathological physiology. Furthermore, it is possible that the number of inciting and/or perpetuating factors may increase or decrease as time goes on so that in some patients only one, while in other patients a combination of factors, may be acting to sustain the elevation of arterial pressure. Therefore, in order to lower the pressure in some patients it may be necessary to correct only one of these factors, while in others it may require a number of different therapeutic procedures applied successively or simultaneously.

It long has been recognized that every psychic tendency seeks adequate bodily expression and that inner tension which cannot be released through ordinary channels may manifest itself in the circulatory system by the response of hypertension. Stewart Wolf,[4] Weiss and English,[4] and others have demonstrated that latent hostility and repressed aggression may cause hypertension, which is improved or may disappear entirely following ventilation of the latent hostility and by understanding or airing of the repressed aggression. Thus, anxiety may be closely related to hypertension.

It has been said frequently that one of the worst possible disfavors to the hypertensive patient was the introduction of the sphygmomanometer into medicine. There is little doubt that the public has become so blood pressure conscious that they can be scared into hypertension by the "alarm reaction" on the physician's face or the thoughtless question, "Has anyone in your family died of high blood pressure?" It has been recognized for decades that the initial pressure taken in the physician's office or in the hospital may be elevated purely on the basis of apprehension. This is doubly true if the physician has an abrupt manner or is hurried or unsympathetic in his approach to the patient. An understandable reaction on the part of the patient to a particularly aggressive physician or nurse is an instantaneous, and at times sustained, pressor reaction of his entire central and autonomic nervous systems. In attempting to offset this reaction, it is wise to point

out to the patient that there is little significance to the level of hypertension since some patients have hypertension for twenty to forty years without any interference with their normal existence. Depending on the physician's knowledge of his patient, it may be unwise for him to acquaint the patient with the fact that hypertension only becomes objectively troublesome when it is complicated by failure in the retinal, cerebral, coronary, or renal circulation. We feel quite certain that there must be many hypertensives, the majority of them females but a fair number of them males, who are asymptomatic and getting along quite well because they have not been subjected to the sphygmomanometer.

For variable periods of time, George Crile,[8, 9] Adson and Craig,[2] Page and Heuer,[26] and Peet,[28] among many other surgeons, were enthusiastic regarding the surgical treatment of hypertension. Smithwick[37, 38] has been the more recent apostle of this form of therapy. As a result of almost twenty years of this combined surgical enthusiasm for sympathectomy, literally thousands of patients have been more or less completely separated from their autonomic nervous systems, if not always from their hypertension. It cannot be denied that out of all of this mutilation of the sympathetic nervous system there emerges occasionally a "miraculous cure," but we have observed none in our series of fifty-two patients followed postoperatively for periods of five to fifteen years. All too frequently we have had the experience of seeing patients operated upon elsewhere and declared "cures" whose pressure, when we measured it later, was extremely high, often as high as the sphygmomanometer could register. This does not indicate dishonesty on the part of our surgical colleagues but it does mean, quite frequently, that these patients drift away from the surgeon and are followed for longer periods of years by the internist or the family physician.

Case Report

Each time I am tempted to become enthusiastic about a new and untried form of surgical or medical therapy, I remember the rather sizable group of patients who have refused surgical treatment for their hypertension and have been unwilling to accept any extreme form of treatment. From this large group I wish to record a patient whose course has defied in every respect the expected outcome of her disorder.

B.C.H., a 37-year-old graduate nurse was first seen by me in 1936 within several days after an ophthalmologist, in checking her eyes for new glasses, had told her that she had "the eyegrounds of a 70-year-old." This obviously was quite upsetting to the patient because her mother and father, two brothers, and a sister had died under the age of forty of complications of hypertension. Her blood pressure at that time was 160/90, but the remainder of her examination was negative. Her chest X-ray was normal for heart size and shape and her E.K.G. was normal. Later that year at the time of surgical treatment for a calcified bursitis her blood pressure was found to be 190/100. Because of her very significant family history of hypertension she was offered sympathetic resection but this she refused. One year later (1937) she experienced a

mild mental depression and her pressure remained at a constant level of 220/120. In 1938, having recovered from her depression, a new position was found with a lesser degree of physical work and a decreased amount of emotional pressure. With this new position, very mild sedation, and frequent opportunities for her to talk over her problems, her pressure fell gradually to 160/90 in the next six years. During the past eight years since 1944, her pressure has ranged from 140/90 to 150/90 with several readings having been found as low as 130/85. She works regularly and has been quite unrestricted in her activities during the past ten years and is now fifty-three years of age. Her heart is normal size and her E.K.G. is normal; her renal function is normal and there have been no hemorrhages or exudate in the eyegrounds.

Study in Progress

We at the present time are in the process of carefully selecting another group of forty to fifty patients who have not been offered surgery or who have refused surgery. We are attempting, in so far as it is possible, to have this group parallel the surgically treated group in age, sex, level of hypertension and proportional number with "malignant" hypertension.

Conclusion

Despite our youthful enthusiasm at the time of our previous report in 1941,[20] we stated that our surgically treated hypertensive patients were "too few to have any statistical significance" and concluded that "the surgical treatment of hypertension, by means of bilateral subdiaphragmatic extraperitoneal resection of the splanchnic nerves, celiac ganglion, and the upper three or four lumbar sympathetic ganglions, seems to give palliative results rather than effecting a cure." Now, eleven years later, as we report on the follow-up of the survivors among the original twelve patients and add forty more patients who were surgically treated by the more extensive thoracolumbar sympathetic resection, between 1940 and 1947, we can still say that the surgical therapy of hypertension, in our experience, does not offer a cure. We can also add sadly that, in our opinion, "the game is not worth the candle"!

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PURPURA VARIOLOSA

Its Manifestations in Skin and Blood*

JAMES W. HAVILAND†

Smallpox in its endemic form in this country (variola minor or alastrim) is seen sporadically and as a rather mild disease. Variola major, on the other hand, is a serious epidemic disease, but fortunately is experienced rarely within the continental limits of the United States. In fact, in recent years it has occurred so infrequently that the experience gained in one outbreak is generally lost or memory of it has faded so completely as to be quite useless by the time the next outbreak is encountered. One of the more severe, but little appreciated, forms of variola major is true hemorrhagic or "black" smallpox (purpura variolosa) with a reported mortality rate of 100%. During the early part of 1946 the Seattle-King County area of the state of Washington had the misfortune to experience an outbreak of 65 cases of variola major with 20 deaths, among which were included the six cases of purpura variolosa which form the nucleus for this paper. Since the epidemiological and clinical details of this outbreak have been given elsewhere,[5, 6, 14] suffice it to say that the smallpox virus was introduced into a susceptible population by a soldier returning from the Orient. To those of us who were caring for the clinical cases of smallpox, purpura variolosa constituted a real diagnostic problem. The observations to be set forth were made by us‡ and are not new, for the clinical descriptions of smallpox made three-quarters of a century and more ago are surprisingly complete and accurate, but the present observers' lack of actual clinical experience with smallpox in its severe classical form and the paucity of pertinent observations in the recent medical literature with regard to this problem have made it seem desirable to set forth our experiences with true hemorrhagic smallpox in some detail.

In order to appreciate purpura variolosa, it is necessary to visualize and emphasize the extraordinarily orderly progression of the clinical course of smallpox. It will be recalled that one of the distinguishing characteristics

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of variola is the singleness of age and development of the skin lesions, which inexorably follow a relatively invariable "time-table":

1. Incubation period — 7-16 days
2. Prodromal period — 3-4 days
3. Eruptive period
   a. Macular stage — 1 day
   b. Papular stage — 1 day
   c. Vesicular stage — 2 days
   d. Pustular stage — 2 days
4. Convalescent period
   a. Crusting stage — several weeks

In addition to the classical forms of discrete and confluent smallpox which conform to the above "time-table," two types of hemorrhagic smallpox have been described—purpura variolosa and variola hemorrhagica pustulosa. These two varieties of hemorrhagic smallpox can be distinguished one from the other with relative ease, since they appear at opposite ends of the "time-table." Pustular hemorrhagic smallpox (variola hemorrhagica pustulosa) in our experience became evident rather late in the course of the disease when hemorrhages were observed to occur in the already developed vesicles and pustules. This form of disease constituted no problem in recognition and will not be considered further at this time. True hemorrhagic smallpox (purpura variolosa), on the other hand, occurred during the prodromal period and was so severe that the patient was dead by the time the onset of the conventional eruption might ordinarily have been expected, that is, by the third to the fifth day of the disease; and therein lay the difficulty in recognition of the condition, smallpox patients succumbing before actual onset of the "pox."

It is easier to view purpura variolosa in its true relationship to classical smallpox if it is remembered that during the prodromal period, accompanying the intense malaise so characteristic of variola major, there frequently occurs a leukopenia, a transient scarlatiniform, morbilliform or petechial rash, and erythema. In cases of true hemorrhagic smallpox, this erythematous eruption, instead of fading, persists and becomes widespread and "lobster-like." Hemorrhages appear and extensive, deep-plum-colored areas are soon seen. The temperature is elevated moderately (101-102° F.). The patient becomes delirious but with effort can be aroused to a state of relative mental clarity and acuity until shortly before death when coma supervenes and death rapidly ensues, frequently to the accompaniment of extensive bleeding not only from the skin but also from the mucous membranes, the gastro-intestinal and the genito-urinary tracts. Studies of the peripheral blood show marked thrombocytopenia and granulocytopenia. It is as though all the concentrated fury and virulence of the smallpox virus

were pent-up, and then explosively and overwhelmingly poured forth on the unsuspecting patient—like an ambush in which the battle is over almost before it is joined, so great is the initial massacre. The clinicians of other generations recognized the grave prognostic significance of the early appearance of this "lobster-like" erythema followed by the black or plum-colored skin hemorrhages, and clearly warned in their writings of the inevitably fatal outcome.

With this recapitulation of the clinical course and prognosis of purpura variolosa in mind, we can proceed to brief summaries of the clinical histories of the six cases of true hemorrhagic smallpox which were encountered in the 1946 outbreak in Seattle.

#207948—M.J., 72 MWW. Admitted on 13 March 1946 in an incoherent state. The only history obtainable indicated that he had been sick for four days with shortness of breath, and that he was probably a diabetic. The temperature was 109° F., the pulse 100, and the blood pressure 130/70. The respirations were of the Cheyne-Stokes variety. The pharynx and uvula were red and edematous. A blowing systolic murmur was described in the mitral area. Hemorrhagic areas were noted about the ankles and scrotum. A fresh vaccination scar was present on the right arm. Soon after admission slightly papular hemorrhagic areas appeared on the arms and hands. These increased greatly in extent and became bluish red. Despite oxygen and penicillin, the patient expired about 15 hours after admission, before blood counts had been obtained. The urine was negative for sugar, acetone, and diacetic acid; a blood culture was positive for staphylococci.

#196921—Z.B., 35 FWW. Admitted on 23 March 1946 because she believed she was coming down with smallpox. The patient was a relative of the King County family in which the first contact case with the original soldier had occurred, and there had been definite contact by the patient with clinical smallpox. She had been vaccinated on 12 March 1946 and developed a primary vaccination reaction. On 21 March 1946 she developed a low backache for which she sought medical attention at King County Hospital on 22 March 1946, but since nothing definite could be found on examination, she was not admitted until 23 March 1946. She was three and a half months' pregnant. The temperature was 99° F. The only positive physical findings were inflamed conjunctivae and a "very ruddy" complexion. Several hours after admission the patient passed a dead foetus. Her backache became very severe. She developed extensive purpura, had several hemoptyses, and exhibited oozing of blood from her mucous membranes. She expired suddenly about 36 hours after admission. No laboratory work was reported.

#202443—L.P., 36 MWD. Admitted on 26 March 1946 because he had developed a rash. This patient had had several outpatient visits because of a fractured femur sustained in December 1945. On 16 March 1946 he had been vaccinated against smallpox and the notation was made that there was no history or evidence of previous vaccination. On 23 March 1946 he had a temperature of 101.6° F. and felt "like the flu." On 25 March 1946 a finely papular rash was noted which may have been somewhat vesicular. On admission he was found to have a primary vaccination reaction. There was an erythematous macular rash over most of the body. By 28 March 1946 the erythema had become much more intense and hemorrhagic areas were developing in the skin and conjunctivae. The patient suddenly became "toxic" on that day and rapidly expired. Laboratory findings—Hgb. 15.9 gms., RBC 5.0 million, WBC 23,900 with 16% polymorphonuclear cells, 78% lymphocytes, 2% eosinophils, and 4% nucleated RBC.

Reticulocytes, 0.1%. Platelets, 10,000. Bleeding time, 1 minute. Clotting time, longer than 10 minutes. Prothrombin time, sample did not clot.

#207394—H.H., 32 FWM. Admitted on 27 March 1946 because of a petechial eruption. The patient had had "flu" early in March 1946 and had come to the Outpatient Department of the King County Hospital with a relative on 12 and again on 20 March 1946. On 24 March 1946 she began to vomit and developed a severe frontal headache and intense low backache. On 26 March 1946 she noted redness of her skin and consulted two private physicians who were unable to find anything of note. On 27 March 1946 her physician first noted a petechial eruption on the trunk, vaccinated her against smallpox for the first time in her life, and sent her to King County Hospital Isolation Ward. P.E. T-97° F., P-108, R-20, BP-134/50. The patient was alert, talkative, and oriented. There were numerous scleral and conjunctival hemorrhages, as well as extensive areas of petechial hemorrhages in the mouth and throat. Widely over the entire skin surface there extended a diffuse, bright erythema with numerous petechial hemorrhages. Laboratory findings—Hgb. 15.3 gms., RBC 3.7 million, WBC 20,500 with 19% polymorphonuclear cells, 79% lymphocytes, and 1% each eosinophils and basophils. Reticulocytes, 0.6%. Platelets, 11,100. Prothrombin time, 61% of the control. Bleeding time, 30 seconds. Clotting time, 6 minutes. The patient received calcium gluconate, ascorbic acid, Vitamin K, desoxycorticosterone acetate, Eschatin, and two blood transfusions. The hemorrhagic diathesis continued to progress. A notation was made by the Resident that the patient was alert and talkative when seen at 7:50 A.M. She expired at 7:55 A.M., some 17 hours after admission.

#56694—J.C., 62 MWD. Admitted on 5 April 1946 in a comatose state from the City Jail where he was noted to have developed a rash. No history was obtained, but the record showed numerous previous admissions to the County Hospital, and recently he had visited the Outpatient Department several times with various complaints. There was no history of previous vaccination. P.E. T-101.4° F., P-88, BP-50/30. There was a diffuse macular eruption superimposed on a diffuse, very bright erythema. Subsequently there appeared extensive hemorrhagic areas on the legs and in the conjunctivae. Despite oxygen therapy and Eschatin, the patient expired without regaining consciousness, some 18 hours after admission. Laboratory findings—Hgb. 12.0 gms., RBC 4.0 million, WBC 12,200 with 37% polymorphonuclear cells, 60% lymphocytes, and 3% monocytes. Platelets, 24,000.

#208241—M.G., 46 FWM. Admitted on 22 April 1946 in a moribund state. On 17 April 1946 the patient had developed a rash which was diagnosed as measles by her private physician who gave her penicillin. During the next few days she complained of severe backache and headache, vomited, and had chills and fever. The rash became hemorrhagic on the day of admission and the patient became unresponsive. There was no known contact with smallpox but the patient lived about three blocks from King County Hospital and had never been vaccinated against smallpox. P.E. T-100° F., P-88, R-20, BP-120/50. The patient was critically ill, drowsy, and mentally unclear. There was an extensive, brightly erythematous, hemorrhagic rash over most of the body. The sclerae and conjunctivae were purple in color from hemorrhages. The tongue, buccal mucous membranes, palate, and pharynx exhibited extensive ecchymotic areas. The liver was palpable three fingers' breadth below the costal margin. Laboratory findings—Hgb. 11.3 gms., RBC 3.8 million, WBC 17,400 with 3% polymorphonuclear cells, 46% stab forms, 4% metamyelocytes, 1% myelocytes, 3% promyelocytes, 36% lymphocytes, and 6% nucleated RBC. Platelets, 11,400. Prothrombin time, 89% of the control. The patient received Vitamin K, ascorbic acid, Eschatin, penicillin, and two blood transfusions. She seemed to improve clinically although the hemorrhagic areas became larger, but she expired suddenly less than 18 hours after admission.

In Table 1 are collected the results of blood studies in the cases which survived long enough to permit the necessary studies to be performed. Unfortunately, bone marrow studies were not done.

The first case observed was thought to have acute lymphocytic leukemia or fulminating meningococcemia (Waterhouse-Friderichsen syndrome). It was not until post-mortem examination with resultant scrutiny of the microscopic sections, which revealed no suggestion of either of these conditions, that the true diagnosis was learned. Subsequent cases were recognized as purpura variolosa but the differential diagnosis was always difficult, and confirmation of the diagnosis of smallpox frequently rested on failure to find changes at post-mortem in the bone marrow or other organs

TABLE 1

PERIPHERAL BLOOD STUDIES—PURPURA VARIOLOSA

		Differential										
Case	*WBC*	*Polys*	*Stab*	*Young Forms*	*Lymphs*	*Monos*	*Eos*	*Basos*	*Nuc. RBC*	*Platelets*	*Hgb.*	*RBC*
J.C.	12,200	37			60	3				24,000	12.0	4.0
L.P.	23,900	16			78		2		4	10,000	15.9	5.0
H.H.	20,500	19			79				6	11,400	11.3	3.8
M.G.	17,400	3	46	8	36		1	1		11,100	15.3	3.7

which would have resulted in establishing some other specific diagnosis, such as leukemia or thrombocytopenic purpura.

When the first case received its correct diagnosis, perusal of the standard textbooks of medicine gave scant help. Brief mention of the rash and sometimes of the blood changes was made but nothing very definite to suggest the diagnosis of purpura varioloa. In VanRooyen and Rhodes[17] was found the first clue to previous experience with this desperate clinical situation. Bancroft,[1] Ker,[10] and Curschmann[3] gave excellent descriptions of the various forms of clinical smallpox, including vivid portrayals of the hemorrhagic varieties. The reports of other outbreaks of the disease generally have carried only a paragraph or two telling of the cases of hemorrhagic smallpox encountered.[4, 11, 13, 16, 19] Weber and Douglass[18] described ten cases of hemorrhagic smallpox seen in a previous outbreak in Seattle but made no mention of the blood picture.

At the time these cases were seen, Ikeda[7, 8, 9] had given the only recent complete description of the blood changes and other remarkable findings in purpura variolosa which could be found in the English literature. In these excellent reports he records not only his own observations during an outbreak of 1,200 cases of smallpox in Minneapolis, but he also quotes Simon[15] to the effect that the platelets are decreased markedly in all forms of smallpox from the prodromal through the vesicular stage, following which they rise rapidly to normal. "In the purpuric form, however, there is a consistent

fall until its termination." Ikeda also noted that there was a consistent leukocytosis in purpura variolosa with a sharp drop, relative and absolute, in the polymorphonuclear leukocytes. Little change in the level of hemoglobin or red blood cells was noted in the fulminating cases under discussion. The appearance in the peripheral blood of pathological forms of normoblasts, basophilic stippling, and polychromatophilia without clinical or laboratory evidence of severe anemia or chronic sepsis was noted, as well as the presence of fragments of condensed nuclear bodies, usually surrounded by a small portion of neutrophilic cytoplasm, indicating the destruction, probably by a toxin, of the neutrophilic polymorphonuclear leukocytes. Marked secondary infection of the skin with accompanying streptococcic bacteremia was found. Ikeda felt that some circulating toxin caused disintegration of the polymorphonuclear cells and that this was followed by a compensatory rise in circulating lymphocytes and young forms of the granulocytic series in response to the defense demands of the body. Magrath, Brinckerhoff, and Bancroft[12] had the opportunity of making extensive blood studies during an epidemic of smallpox in Boston in 1902-03 and their careful studies are in substantial agreement with the previously mentioned studies of Ikeda.

Treatment with antibiotics, transfusions, adrenal cortical extracts, and other supportive therapy was completely without discernible benefit. The mortality rate in our six cases was 100%. Post-mortem examination of three of these patients showed tissue changes similar to those described in standard textbooks of pathology.

Discussion

It was our impression that the dramatic blood changes and skin manifestations of purpura variolosa resulted from the severe "toxemia" of an overwhelming infection with smallpox virus in a susceptible host, for careful studies failed to confirm bacteriological or histological findings, made in pre-antibiotic times, which led to the conclusion that death was due not only to smallpox virus, but also to extensive secondary bacterial invasion.

Our difficulties in recognizing this clinical condition are well expressed in the following quotation from Councilman: "It is doubtful if a sporadic case could be correctly diagnosed, particularly if the physician were unacquainted with (hemorrhagic) smallpox."[9] These difficulties stemmed from two sources, in the main: (i) lack of familiarity with purpura variolosa with resultant failure to appreciate the severity of this fulminating illness and its attendant clinical pathology, and (ii) failure to include smallpox as an item worthy of consideration in the differential diagnosis of purpura. Furthermore, although there has been careful documentation of the blood changes and clinical course of hemorrhagic smallpox, the majority of these descriptions have appeared in the rather distant past and have not been readily accessible to the present-day physician. Consequently, it has seemed

appropriate to set down our experiences with hemorrhagic smallpox and to reaffirm the outstanding blood changes and the striking clinical course of this condition in the light of this era of antibiotics and chemotherapy. Purpura variolosa is still an invariably fatal disease, the correct diagnosis of which may not be suspected ante-mortem, but frequently must await post-mortem confirmation.

Conclusions

1. Purpura variolosa (true hemorrhagic smallpox) is a rapidly and invariably fatal illness which occurs during the prodromal and early eruptive periods of the disease.

2. The skin changes clinically are characterized by petechial and purpuric hemorrhages, generally extensive, of black or deep plum color, superimposed on a bright erythema best described as "lobster-like" in appearance.

3. Thrombocytopenia and leukocytosis with granulocytopenia, young forms of erythrocytes, basophilic stippling, and polychromatophilia are the characteristic blood findings.

4. Purpura variolosa must be considered in the differential diagnosis of acute purpura.

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THE ACTION OF CRYSTALLINE TRYPSIN AND SOYBEAN TRYPSIN INHIBITOR ON THE CLOTTING OF BLOOD BY STAPHYLOCOAGULASE*

MORRIS TAGER†

The mechanism of the coagulation of blood by staphylocoagulase and the relation of this process to physiological clotting present many unsolved problems. Evidence has been advanced for the concept that coagulase reacts with a plasma component (coagulase reacting factor CRF) which is distinct from prothrombin.[5, 14, 16–18] The formation of an intermediate complex has been inferred from the shortening of the clotting time when some preparations of coagulase and plasma are incubated before the addition of fibrinogen.[14, 16] A simplified representation of the reaction may thus be assumed to proceed along these lines:

Coagulase plus CRF = intermediate complex
Intermediate complex plus fibrinogen = fibrin

The evidence for the differentiation of CRF from prothrombin has been presented elsewhere.[16–19] That this concept has not been accepted without reservations is evident from the recent studies of Duthie and Lorenz,[3, 4] who are not entirely convinced that prothrombin and CRF are separate entities and reconsider the possibility that CRF and prothrombin may be identical, or that CRF may be a modification of prothrombin. With regard to the formation of the "intermediate complex," while a shortening of the coagulation time when coagulase and plasma are incubated has been frequently noted, incubation of some preparations of plasma and coagulase, in past (unpublished) studies, failed to produce any significant acceleration of clotting.

These problems of coagulase clotting have prompted the present study of the action of trypsin and its inhibitor on this system. The acceleration of physiological clotting by trypsin has been recognized for many years,[2, 5] and more recently, the inhibitory capacities of trypsin inhibitors, such as the soybean trypsin inhibitor of Kunitz have been established.[1, 7, 8, 12, 23] The isolation of these agents in crystalline form[10, 11] has helped to resolve earlier conflicts[13] which stemmed from the use of impure reagents in improper amounts. The primary purpose of the present study, therefore, was to

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investigate the action of the trypsin and its inhibitor on coagulase clotting, with the object of possibly disclosing some differences from, or similarities to, the mode of action of these agents on physiological clotting. The use of the enzyme inhibitor was further suggested as a means of investigating the mechanism of CRF loss when blood coagulates. In a recent study,[19] this CRF consumption has been shown to depend on prothrombin conversion. Since this reaction constitutes a possible link between the coagulase and the physiological clotting systems, and since there is evidence that proteolysis occurs during prothrombin conversion, the effect of the tryptic inhibitor on this reaction seemed worthy of study.

Materials and Methods

Coagulase—a partially purified cell-free product derived from *Staphylococcus aureus* #104[15] was used throughout.

Plasma—dilutions of citrated human blood bank discards were used as the source of CRF.

Fibrinogen—Armour's bovine Fraction I was employed in a concentration of 200 mgs. per cent in veronal buffer at a pH of 7.2.

Trypsin—twice crystallized lot TR 340 (Worthington Biochemicals) was dissolved in 0.9% saline, and was dialyzed against 1/1000 M HCl; 150 mgs. of the original material was estimated to contain 60 mgs. of protein.

Trypsin inhibitor five times crystallized—Soybean trypsin inhibitor (SBTI), lots S 55 and S 62 (Worthington Biochemicals) were dissolved in M/15 phosphate buffer at a pH of 7.1, and subdilutions were made in physiological saline.

All the above reagents were made up in small lots, and maintained in the deep freeze unit at about —15° C.

General method of determining the clotting time. Tests were made in pyrex glass tubes 7 × 12 mm., using a 2x magnifying lens and a constant fluorescent light source. The time of the appearance of the first wisp of fibrin or of fine floccules was taken as the clotting time and was recorded to the nearest second with a stopwatch. Further details of procedures are considered under Results.

Results

The effect of crystalline trypsin on the coagulase clotting of plasma. Previous studies have demonstrated that crude and crystalline trypsin will inactivate coagulase.[20, 24] For the present purposes, therefore, minute amounts of trypsin were selected which produced no evident fall in coagulase titer during the period of observation. Both the plasma and the trypsin dilutions were made in citrated saline. Trypsin at the concentrations indicated in Table 1, the plasma dilution, and coagulase were mixed in equal volumes, and 0.2 ml. samples were withdrawn and reacted with 0.2 ml. of fibrinogen at the stated times. The potentiating effect of 1 microgram and of 0.5 micrograms of trypsin was readily apparent, while 0.1 micrograms/ml. of trypsin was without effect. Even this minute amount, however, accelerated physiological clotting (Table 2).

In subsequent experiments, heparin in excess was also added to the plasma before reacting with trypsin and coagulase. The same acceleration of clotting in the test system was noted, while no clotting was noted in the trypsin-plasma controls at any of the levels observed.

TABLE 1

POTENTIATION OF COAGULASE CLOTTING BY TRYPSIN

Test no.	Incubation mixture*			Clotting time in min. & sec. at				
	Trypsin†	Plasma‡	Coagulase	0	10′	20′	30′	40′
1	1	0.5	0.5	7′ 30″	4′ 1″	2′ 30″	2′ 20″	2′ 40″
2	0.5	0.5	0.5	7′ 43″	6′ 6″	3′ 56″	3′ 43″	4′
3	0.1	0.5	0.5	11′	8′ 40″	6′ 51″	6′ 28″	6′ 17″
4	0	0.5	0.5	10′ 55″	8′ 45″	6′ 47″	6′ 33″	6′ 33″
5	1	0.5	0	60–120′	60–120′	60–120′	60–120′	60–120′
6	0.5	0.5	0	0	0	0	0	0
7	0.1	0.5	0	0	0	0	0	0

* All volumes made up to 1.5 ml.
† Final concentration in micrograms/ml. in the incubation mixture.
‡ Plasma diluted 1 : 50 in saline and 0.3% sodium citrate.

TABLE 2

THE EFFECT OF TRYPSIN ON PHYSIOLOGICAL CLOTTING

Test no.	Prothrombin conversion mixture*			Clotting time in min. & sec. at				
	Trypsin†	Ca (0.02M)	Plasma‡	0	15′	30′	1°	1° 30′
1	1	0.5	0.5	16′ 30″	4′	2′ 52″	2′ 09″	2′ 04″
2	0.1	0.5	0.5	45′	10′ 42″	8′ 25″	1′ 25″	1′ 24″
3	1	0	0.5	1° 20′	25′	15′	10′	
4	0.1	0	0.5	No clot at 1½°	No clot	No clot	No clot	No clot
5	0	0.5	0.5	1° 15′	1° +	45′	2′ 30″	1′ 2″

* All volumes made up to 1.5 ml.
† Final concentration in micrograms/ml. in the incubation mixture.
‡ Plasma diluted 1 : 50 in saline.

The effect of SBTI on the clotting of blood by coagulase. The acceleration of both physiological and coagulase clotting by trypsin prompted a study of the activity of the tryptic inhibitor (SBTI) on the coagulase-CRF reaction. The inhibition of physiological clotting by SBTI has been repeatedly demonstrated although the site of action is not definitively established. As little as 2.5 micrograms/ml. have been shown to exert an inhibi-

tory effect on thrombin formation.[8] Preliminary titrations indicated that the incubation of SBTI and coagulase for 1 hour at 37° C. failed to exert any effect at levels exceeding 1000 micrograms/ml. Only very high concentra-

TABLE 3

THE EFFECT OF SBTI ON CLOTTING OF BLOOD BY COAGULASE

Test no.	*Incubation mixture**			*Clotting time in min. & sec at*			
	SBTI†	*Coagulase*	*Plasma*‡	0	15′	30′	60′
1	6600	0.5	0.5	7′ 40″	5′ 15″	3′ 55″	4′ 5″
2	660	0.5	0.5	6′ 57″	4′ 30″	4′ 31″	4′ 8″
3	66	0.5	0.5	6′ 40″	4′ 25″	4′ 25″	4′ 16″
4	6.6	0.5	0.5	6′ 10″	4′ 13″	4′ 13″	4′ 2″
5	0	0.5	0.5	6′ 30″	4′ 45″	4′ 45″	4′ 7″
6	6600	0	0.5	0	0	0	0
7	660	0	0.5	0	0	0	0
8	66	0	0.5	0	0	0	0
9	6.6	0	0.5	0	0	0	0

* All volumes made up to 1.5 ml.
† Final concentration in micrograms/ml. in the incubation mixture.
‡ Plasma diluted 1 : 50 in saline and 0.3% sodium citrate and 0.1 mg. heparin/ml.

TABLE 4

THE NEUTRALIZATION BY AN EXCESS OF SBTI OF THE EFFECT OF TRYPSIN ON THE COAGULASE CLOTTING OF PLASMA

Test no.	*Incubation mixture**					*Clotting time in min. & sec. at*			
	Trypsin†	*SBTI*	*Trypsin & SBTI*‡	*Plasma*¶	*Coagulase*	0	10′	20′	30′
1	1	0	0	0.5	0.5	7′ 40″	3′ 13″	2′ 41″	2′ 36″
2	0	250	0	0.5	0.5	7′ 25″	4′ 50″	4′ 44″	4′ 50″
3	0	0	0.5	0.5	0.5	7′ 05″	5′	5′ 10″	4′ 25″
4	0	0	0	0.5	0.5	8′ 10″	5′ 25″	5′ 25″	4′ 50″
5	1	0	0	0.5	0	0	0	0	0
6	0	250	0	0.5	0	0	0	0	0
7	0	0	0.5	0.5	0	0	0	0	0

* All volumes made up to 1.5 ml.
† Final concentration in micrograms/ml. in the incubation mixture.
‡ Mixture of equal parts of SBTI and trypsin to give final concentration of 1 microgram of trypsin/ml. and 250 micrograms of SBTI/ml.
¶ Plasma diluted 1 : 50 in saline and 0.3% sodium citrate and 0.1 mg. heparin/ml.

tions, such as 6,000-12,000 micrograms/ml. produced some prolongation of the clotting time. Attention was, therefore, directed towards evaluating the possibility that SBTI may interfere in the coagulase-CRF reaction. Accordingly, equal volumes of various concentrations of SBTI, of suitable plasma dilutions, and of coagulase were mixed, and 0.2 ml. of the incubation mixture added to 0.2 ml. of fibrinogen at various time intervals. It will be noted

(Table 3) that the coagulase clotting system proved highly refractory to the action of SBTI. Only at excessively high levels is there a delay in the initial clotting time—comparable to the effect on preincubating the inhibitor and coagulase—while the shortening of the coagulation time upon incubation of coagulase and CRF is not blocked even at these excessive levels of inhibitor. In subsequent experiments, modifications of the above procedure were explored in seeking conditions which might reveal a significant effect. Dilutions of plasma, from 1 : 10 to 1 : 1000 were tested against a range of coagulase dilutions, as well as plasma samples which had been subjected to five successive Seitz filtrations to remove prothrombin activity. Neither was any significant effect on the initial clotting time noted except at the very high levels of inhibitor, nor was the shortening of the clotting time follow-

TABLE 5

THE EFFECT OF SBTI ON CRF LOSS AFTER PROTHROMBIN CONVERSION

Test no.	*Prothrombin conversion incubation mixture**				*Residual CRF clotting time at final serum dilutions of*	
	SBTI†	Ca (0.02M)	*Thrombo-plastin*	*Plasma*	1 : 30	1 : 150
1	4000	0.2	0.2	0.2	2′ 05″	5′ 30″
2	400	0.2	0.2	0.2	2′ 21″	5′ 47″
3	40	0.2	0.2	0.2	3′	7′ 35″
4	4	0.2	0.2	0.2	3′ 57″	8′ 36″
5	0.4	0.2	0.2	0.2	3′ 54″	8′ 20″
6	0.04	0.2	0.2	0.2	4′ 6″	9′ 04″
7	0.004	0.2	0.2	0.2	4′ 9″	10′ 19″
8	0	0.2	0.2	0.2	4′ 54″	12′ 36″
9	0	0	0.2	0.2	1′ 35″	3′ 30″

* All volumes made up to 1 ml. with veronal buffer.
† Final concentration in micrograms/ml. in the incubation mixture.
Thrombin controls on test, by omitting coagulase: no clots in 1 hour.

ing the incubation of coagulase and CRF blocked at any level of inhibitor tested. In a further titration, a great excess of SBTI was added to trypsin at a final concentration of 1 microgram/ml. and then reacted with coagulase and plasma. The superimposed accelerating effect of trypsin was abolished, but not the base-line speeding up of the reaction such as occurred in the controls (Table 4).

The effect of SBTI on the CRF loss incurred during prothrombin conversion. It has been shown that the conversion of plasma to serum results in a loss of CRF.[3, 4, 19] and that conditions which favor the most effective prothrombin conversion bring about maximal CRF loss. Since SBTI interference with the physiological coagulation presumably involves prothrombin conversion, it seemed indicated to determine whether interference with proteolysis would spare CRF. In the experiment summarized in Table 5,

plasma was converted by calcium and thromboplastin (rabbit brain), and various concentrations of SBTI were incorporated in the conversion mixture. After coagulation occurred, the clots were shaken loose, and the samples incubated at 37° C. for two hours to allow antithrombin to form. The sera were then diluted, and aliquots titrated for CRF by the addition of coagulase and fibrinogen. It will be noted that as little as 0.4 micrograms/ml. of SBTI (#5) partially blocked CRF loss, while the effect of the further dilutions of the SBTI approached the conversion control (#8). On the other hand, even at the maximal concentration of SBTI tested, 4000 micrograms/ml. (#1), the residual CRF titer did not attain the level of the sample in which no prothrombin conversion occurred (#9). Subsequent experiments were confirmatory of these findings. Finally, the removal of directly demonstrable prothrombin activity by five successive Seitz filtrations abolished CRF loss regardless of the addition of calcium, thromboplastin, or SBTI.

Discussion

Many observations have favored the distinctiveness of coagulase clotting from the physiological process. Among these may be mentioned the failure of citrate, oxalate, and of heparin to interfere with coagulase activity. Highly purified bovine prothrombin of Seegers is all but completely devoid of CRF even when reacted with coagulase preparations of high potency and purity.[15] The dissociation of prothrombin activity from CRF by serial Seitz filtration also supports this concept.[18]

That many of the arguments admit of alternative interpretations cannot be denied. Thus, the activation of prothrombin by systems other than calcium and "thromboplastin" is receiving wide recognition. It is known that Seitz filtration does not eliminate all prothrombin activity,[4, 18] and that, indeed, some additional activity may be regenerated by appropriate chemical procedures from what appear to be prothrombin-free filtrates. Duthie and Lorenz[4] suggest that it may be conceivable that repeated Seitz filtration may actually not remove prothrombin, but that this procedure so alters it that, while it can no longer be converted to thrombin, it still retains its ability to react with coagulase. It is of interest in this regard to note that CRF properties are modified when separation from prothrombin is achieved by serial Seitz filtration,[21] suggesting, though not proving, a possible close relationship between the two agents in the native state. Duthie and Lorenz[4] also interpret parallel CRF and prothrombin loss consequent on prothrombin conversion, as well as the similar effects of dicoumarol administration on prothrombin and CRF, as pointing in the same direction.

The present writer continues to favor the view that prothrombin and CRF are not identical, and that the two clotting mechanisms are distinct. Final solution of the problem awaits adequate purification of CRF. Attempts in this direction have met with only limited success. Although

purification of the order of one hundred and twenty-five-fold has been attained, the products proved highly labile and were not free of slight traces of prothrombin activity.[22]

The studies here reported have afforded a further basis for comparison of the two clotting systems. Both responded to minute concentrations of added trypsin. The coagulase clotting system, however, proved all but completely refractory when tested against excessively high levels of two lots of inhibitor. There was no evidence of any effect on the initial clotting time except at very high levels of SBTI, and the formation of the "intermediate complex" was not blocked at any level of inhibitor tested. The same lots of SBTI, in minute amounts, proved effective in delaying the clotting of citrated plasma by recalcification. If the activation of prothrombin involves proteolytic enzyme systems vulnerable to SBTI, then the coagulase reaction either involves proteolytic enzyme systems of different specificities or proceeds along diverse pathways altogether.

The acceleration of the clotting of plasma by the addition of minute quantities of trypsin to coagulase suggests the need for caution in the interpretation of the formation of an "intermediate complex" when coagulase and CRF are incubated. The presence of proteolytic enzymes in either crude coagulase[6] or in plasma may contribute to such acceleration. However, in the present studies, the order of acceleration resulting from the incubation of partially purified coagulase and diluted plasma was far slighter than the shortening of the clotting time occurring when trypsin was added. It is noteworthy, too, that while an excess of SBTI abolishes the superimposed accelerating effect of trypsin, the base-line acceleration is not disturbed. The suggestion may be entertained that some of the more striking accelerations[14, 16] reported in the literature when coagulase and CRF are incubated may represent a summation of a true "intermediate complex" and the activity of contaminating proteolytic enzymes.

Milstone[15] has presented evidence that the thrombokinase, the activator of prothrombin, is an enzyme, possibly proteolytic. The sparing effect of minute quantities of SBTI on CRF loss after prothrombin conversion—quite within the range of trypsin and trypsin inhibitor activity on physiological clotting—makes it seem acceptable that proteolysis occurring during prothrombin conversion is the mechanism underlying this CRF loss.

Summary

Crystalline trypsin at concentrations of the order of 0.5 to 1 microgram/ml., accelerates the clotting of plasma by coagulase.

Contrary to its effect on physiological clotting, crystalline soybean trypsin inhibitor (SBTI) fails to influence the coagulase clotting process, with the exception that a slight prolongation of initial clotting time takes place at high levels of inhibitor.

SBTI effectively, though not completely, blocks CRF loss incurred during prothrombin conversion.

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A STUDY OF THE CUTANEOUS REACTIONS TO OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS SKIN TEST ANTIGENS IN PATIENTS WITH SARCOIDOSIS*

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A decrease in the reaction to intracutaneous injection of tuberculin or its purified protein derivative is generally accepted as a feature of the disease syndrome sarcoidosis. The phenomenon has aroused considerable interest, and many discussions of the various possible underlying mechanisms have appeared in the literature.[5, 10, 11] A few detailed investigations have been conducted, but there is no information on the delayed reactions of individuals with this disease to commonly encountered antigens other than tuberculin. Reported here are the results of a study of this problem.

METHODS

The reactions to the intracutaneous injection of several antigens were studied in cases of sarcoidosis and in a group of control patients. First and second strength purified protein derivative was used to determine the reaction of each individual to tuberculin. Tests were made at the same time with three other unrelated antigens which produce tuberculin-like reactions: mumps virus, oidiomycin, and trichophytin. The mumps antigen was a preparation of chick embryo allantoic fluid, normal allantoic fluid serving as control test material.[8] *Candida albicans* extract (oidiomycin) was used in a dilution of 1:100 and *Trichophyton gypseum* extract (trichophytin) was used in a dilution of 1:30.‡[1] The antigens were injected in 0.1 ml. doses and the reactions read after 45-50 hours. In reading the tests, the largest diameter of the area of edema was measured. Erythema is also an indication of a positive reaction, but this was not used because it is frequently obscured in dark-skinned individuals. At the time of the skin tests each individual also received a subcutaneous injection of 1.0 ml. of a pneumococcus vaccine.¶ Serological studies on serum obtained at that time and 21 days later will be the subject of a later report.

Tests were carried out on 86 individuals, of whom 64 were controls and 22 were cases of sarcoidosis.§ This diagnosis was considered established if clinical, laboratory,

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‡ The mumps skin test antigen was furnished by the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, through the kindness of Dr. Victor Cabasso, Section of Viral and Rickettsial Research. The oidiomycin and trichophytin were also a product of Lederle Laboratories.

¶ Pneumococcus polysaccharide vaccine A., E. R. Squibb & Sons, Inc., New Brunswick, New Jersey. This preparation contains polysaccharides of types 1, 2, 3, 5, 7, and 8.

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or roentgenological studies revealed evidence of a disseminated disease process, biopsy of one or more sites revealed characteristic epithelioid granulomata, and attempts to establish a specific etiologic diagnosis were not successful. Such studies included stain, culture, and animal inoculation of sputum, and stain for the tubercle bacillus on all biopsy material. The chief clinical features of the cases of sarcoidosis are summarized in Table 1.

TABLE 1

CLINICAL FEATURES OF THE CASES OF SARCOIDOSIS INCLUDED IN THIS STUDY*

	Case 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Lung		x	x		x		x	x	x	x	x		x			x	x	x	x		x	x
Lymph nodes	x	**x**	x	**x**	**x**	**x**		x	**x**	x	x	**x**	x		**x**	**x**	x	x	x	x	**x**	x
Liver	**x**					x		**x**		**x**	**x**	**x**		**x**	x		**x**	**x**	**x**	**x**		**x**
Spleen			**x**					x				x		x	x			x				**x**
Skin		**x**					**x**	x	**x**													**x**
Iridocyclitis	x																x					
Salivary gland									**x**				**x**									
Fever	–	–						x	x				x	x								
Arthralgia									x													x
Respiratory symptoms		x			x				x													**x**
Other	x							x	x				x				x	x	x			
Increased serum globulin	x	x	x	x		x	x	x		x	x		**x**	x	x		x	x			x	x
1st PPD	0	0	0	0	0	0	0	0	0	0	0	0	**0**	0	0	0	1.5	0	0	0	2.0	1.5
2d PPD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.0	..	3.0	1.5	2.0	0	0

* The marks in boldfaced type indicate that biopsy from that particular site revealed the presence of characteristic epithelioid granulomata.

The control group included 29 individuals who presented no evidence of disease, and 35 suffering from a variety of chronic systemic diseases. Cachectic patients, and those acutely or severely ill, were excluded from the study because of the known decrease in skin reactivity in such states. There were at least as many female and Negro subjects in the control as in the experimental group, although the proportionate numbers were smaller in the former. The mean age of the controls was 33 years, and of the sarcoidosis patients 36. Of the controls, 17 per cent were younger than 25 years of age and 14 per cent were older than 44. Corresponding figures for the experimental group were 14 and 23 per cent, respectively. The tuberculin negative control and experimental groups resembled each other more closely in mean age and range of age distribution than did those in the groups that were tuberculin positive. In the control group there were no appreciable differences in diameter or frequency of positive reactions between whites and Negroes, between normal individuals and those with disease, or between those of different ages. However, there was a difference between males and females in the frequency of reactions to trichophytin. Seventy-two per cent of the males and 29 per cent of the females reacted to this antigen. Males and females did not differ, however,

in the mean diameter of positive trichophytin tests, nor did they differ in frequency or mean diameter of reactions to oidiomycin or the mumps antigen. Therefore, in analyses of data involving the frequency of reactions to trichophytin, males and females were considered separately. Statistical analysis was carried out whenever data showed apparent differences, using the t test when mean values were being compared, and the chi-square test when the data were concerned with the frequency of positive tests. Differences which were statistically significant are indicated in the tables by inclusion of the results of the analysis.*

TABLE 2

MEAN DIAMETER OF RESULTS WITH OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS ANTIGEN IN ALL CASES

		Mean diameter of reactions				*Difference and standard error**
			Trichophytin			
	Number tested	*Oidiomycin*	*Males*	*Females*	*Mumps*	
Tuberculin negative	Sarcoidosis 15	0.49 cm. ① ②	0.32 cm. ③	0.00 cm.	0.24 cm. ④	① 1.79 ± 0.58 ② 1.74 ± 0.78 ③ 0.68 ± 0.33 ④ 1.04 ± 0.33 ⑤ 0.85 ± 0.39
	Controls 20	2.28 cm. ①	1.00 cm. ③	0.14 cm.	1.28 cm. ④	
Tuberculin positive	Sarcoidosis 7	2.23 cm. ②	0.76 cm.	(1 case only)	0.24 cm. ⑤	
	Controls 44	2.50 cm.	1.18 cm.	0.50 cm.	1.09 cm. ⑤	

* The encircled numbers relate the results of the statistical analyses to the corresponding values in the table.

Reactions to the normal allantoic fluid material used with the mumps test occurred in 12 of the individuals tested. In no instance was there a reaction to normal allantoic fluid in the absence of a reaction to that containing virus. Of those in whom the reactions to virus and control material were of nearly equal size, none had a history of mumps infection. Of five individuals in whom the diameter of the reaction to the virus was larger than 1.0 cm. and at least three times as large as that of the control, the history of mumps was positive in four. Because of these findings, and the experience of others that positive and negative tests can be distinguished even when the control gives a reaction,[2] these five reactions were accepted as specific positive tests. There were five reactions to the virus that were less than 0.5 cm. in diameter, and because the normal allantoic fluid gave no reaction, these were accepted as positive tests. With oidiomycin and trichophytin a total of three tests gave reactions of less than 0.5 cm. in diameter. Whether these are specific or non-specific reactions cannot be determined, but their inclusion does not alter the significance of the observed differences.

RESULTS

In Table 2 are shown the degree of reactivity to each of the three antigens in the tuberculin negative and positive sarcoidosis cases and

* The statistical analyses were carried out by Dr. M. H. Quenouille, Department of Biostatistics, Yale University.

corresponding controls. These values are expressed as the means of all individuals in each group, whether positive or negative.

The mean diameter of the oidiomycin tests in tuberculin negative sarcoidosis was significantly smaller than it was in either tuberculin negative controls or tuberculin positive sarcoidosis. In the male subjects the diameter of the trichophytin tests in tuberculin negative sarcoidosis was significantly smaller than the tuberculin negative controls. Trichophytin reactions in females with sarcoidosis and in the tuberculin positive males with sarcoidosis tended to be smaller than corresponding controls, but these differences were not significant. Reactions to the mumps antigen were significantly smaller in both tuberculin reactors and non-reactors with sarcoidosis than in the controls.

TABLE 3

THE MEAN DIAMETER OF TESTS IN CASES REACTING TO OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS

Number tested		*Mean diameter of reactions*			*Difference and standard error**
		Oidiomycin	*Trichophytin*	*Mumps*	
Tuberculin negative	Sarcoidosis 15	0.91 cm. ①	0.95 cm.	0.90 cm.	① 1.50 ± 0.72 ② 1.14 ± 0.51
	Controls 20	2.41 cm. ①	1.31 cm.	1.60 cm.	
Tuberculin positive	Sarcoidosis 7	2.60 cm.	1.06 cm.	0.57 cm. ②	
	Controls 44	2.69 cm.	1.55 cm.	1.71 cm. ②	

* See footnote, Table 2.

It was of interest to determine whether similar differences could be demonstrated using data from which the factor of frequency of positive tests had been eliminated. This was done by calculating the mean diameter of all positive tests (Table 3). The difference between tuberculin negative sarcoidosis and controls in the mean diameter of positive oidiomycin tests was statistically significant, and this was also true for reactions to mumps antigen in tuberculin positive sarcoidosis and controls.

The frequency of positive tests in the various groups is shown in Table 4. The same trends that have been evident in previous tabulations are apparent here. In respect to oidiomycin and mumps antigen, the differences between tuberculin negative sarcoidosis and tuberculin negative controls were significant.

It was of some interest to consider the reactions of individuals to all three antigens in addition to the separate results with each. The data presented in Table 5 show the number of individuals reacting to at least two of the three antigens, the number reacting to less than two, and the mean number of positive tests per person. In the control group there were few individuals, whether they reacted to tuberculin or not, who did not react to at least two of the other three antigens. In the experimental group, those who reacted to tuberculin usually reacted to two or more of the other materials, whereas those not reacting to tuberculin seldom reacted to more than one. This

TABLE 4

THE FREQUENCY OF REACTIONS TO OIDIOMYCIN, TRICHOPHYTIN, AND MUMPS ANTIGEN IN SARCOIDOSIS AND CONTROLS

		Number tested	*Number of tests positive*								P*
			Oidiomycin		*Trichophytin*				*Mumps*		
					Males		*Females*				
			No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	
Tuberculin negative	Sarcoidosis	15	8 ①	53	2/6	33	0/9	0	4 ②	27	① < 0.02 ② < 0.01
	Controls	20	19 ①	95	11/15	73	1/5	20	16 ②	80	
Tuberculin positive	Sarcoidosis	7	6	86	5/6	83	0/1	0	3	43	
	Controls	44	41	93	25/35	71	3/9	33	28	64	

* See footnote, Table 2.

difference was highly significant for the male but not for the female group. The mean number of positive tests per person was significantly smaller in the tuberculin negative sarcoidosis cases than in the tuberculin negative controls for both the male and female groups. Males with tuberculin negative sarcoidosis differed significantly from those who were tuberculin positive, but the latter group was similar to the two groups of controls.

DISCUSSION

The reactions to these antigens in patients with sarcoidosis were both quantitatively and qualitatively less than in the controls. This effect was most marked in cases of sarcoidosis with negative tuberculin reactions. Patients with sarcoidosis and positive tuberculin tests had reactions to these antigens which were intermediate between the tuberculin negative cases of sarcoidosis and the controls. It appears that the mechanism

responsible for this altered state of reactivity was most pronounced in the tuberculin negative cases.

Various explanations have been proposed for the decreased tuberculin reactivity in sarcoidosis. The most popular point of view has been the theory of so-called "positive tuberculin anergy" which assumes that the decreased reactivity is to tuberculin alone. This implies that the reaction of such individuals to other antigens to which they may have been previously exposed would be unaltered. The data that have been presented here indicate that the reaction to the other antigens is altered, and that the phenomenon is not specific for tuberculin. Proponents of the theory that the tubercle

TABLE 5

NUMBER OF POSITIVE TESTS PER PERSON IN SARCOIDOSIS AND CONTROLS

Number tested		*Number positive*						P*
		Males			*Females*			
		>1	<2	*Mean*	>1	<2	*Mean*	
Tuberculin negative	15 Sarcoidosis	1 ①	5 ①	0.83 ② ③	2	7	1.00 ④	① < 0.01 ② < 0.01 ③ < 0.05 ④ < 0.05
	20 Controls	14 ①	1 ①	2.43 ②	4	1	2.00 ④	
Tuberculin positive	7 Sarcoidosis	5	1	2.17 ③	..	1	.....	
	44 Controls	30	5	2.26	6	3	2.00	

* See footnote, Table 2.

bacillus is the etiologic agent of sarcoidosis have advanced this "specific tuberculin anergy" as an argument in support of their view.[5, 11] The decreased tuberculin reactivity has also been cited by those opposed to this theory.[6, 9] The evidence presented here indicates that the phenomenon carries no implication in either direction.

Recently it has been shown that in the United States most individuals with sarcoidosis are of southern rural origin, and the suggestion has been made that the decreased incidence of tuberculin sensitivity may be due to a decreased exposure to tuberculosis.[7] If this were true, reactions to the mumps antigen and perhaps also trichophytin might be similarly affected. Although there is no evidence to suggest that infection with *Candida albicans* is less frequent in rural areas, there was a lower frequency of reactions to this antigen among the tuberculin negative members of the experimental group than among the controls. Many of the cases studied here did come originally from rural areas, but most of them had lived in urban areas

for many years, and it seems probable that the effect of rural isolation would have been minimized. Several of the individuals who did not react to any antigens had always lived in urban surroundings. For these reasons it does not seem likely that the differences in frequency of positive tests can be attributed to this factor. Certainly the smaller mean diameter of positive reactions in the sarcoidosis cases cannot be explained on epidemiological grounds.

The results of these tests seem to fit only with the concept of a mechanism which would suppress delayed cutaneous reactions to all antigens. There is actually little to indicate that the abnormality is primarily concerned with immunological reactions rather than the reactivity of tissue to a variety of stimuli. This problem is now under study.[4]

These results also have some practical importance. The tests may be useful in determining the ability of an individual to react, particularly in cases of suspected sarcoidosis. It is apparent that cutaneous sensitivity reactions of the delayed type should not be expected to give reliable information in specific etiologic studies in sarcoidosis.

Summary and Conclusions

In a study of the reactions of patients with sarcoidosis to mumps virus and extracts of *Candida albicans* and *Trichophyton gypseum,* it was found that there was a generalized depression of reactivity as compared with a group of controls. This was greatest in those with negative tuberculin reactions. The implications of these findings in regard to the various etiologic and immunologic theories of the disease are discussed. Certain practical applications of such tests are mentioned.

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THE EFFECT OF DOCA ON ELECTROLYTE BALANCE IN NORMAL MAN AND ITS RELATION TO SODIUM CHLORIDE INTAKE*

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It has been widely accepted that intramuscular administration of synthetic 11-desoxycorticosterone acetate (DOCA) causes urinary retention of sodium, chloride and water, and loss of potassium both in normal subjects and in those with adrenocortical insufficiency.[2, 18, 39] There is, however, a paucity of published information concerning many aspects of this question which have an important bearing on the problem of the adrenocortical regulation of fluid, electrolyte, and nitrogen balance. In an effort to elucidate this problem, detailed balance studies have been conducted on normal young adults who were given 20 to 40 mgm. of DOCA intramuscularly for periods of three to eleven days.

METHODS AND MATERIALS

A total of nine balances were done on seven healthy young adults: medical students, house officers, and technicians. Each study consisted of a pre-treatment control period of from three to twelve days, followed by a treatment period of from three to eleven days during which time an oily suspension of DOCA‡ was administered intramuscularly twice daily. In five studies, post-treatment periods of from four to nine days were included; in the other four it was not possible to continue observations past the end of the treatment period. The subjects were allowed to continue their usual activities during the period of study but were instructed to avoid all forms of vigorous exercise. All the studies were carried out in the winter or early spring months, when skin losses through sweating should be minimal. The subjects were fed constant diets which contained only 13-15 mEq. of sodium and 15-20 mEq. of chloride but which were otherwise normal in composition. Potassium content was 61 to 95 mEq. In three studies, no sodium chloride was added to the diets; in three others approximately 180 mEq. of sodium chloride were added to the diet each day by use of a weighed salt shaker. In the remaining three subjects approximately 40 mEq. of sodium chloride were added in the salt shaker and an additional daily increment of approximately 450 mEq. of sodium chloride was administered intravenously, from a calibrated flask, in the form of three liters of isotonic saline solution. Oral fluid intake was held constant in each of these latter cases at between 1500 to 1700 cc. per day, while the intake of

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other subjects who were not receiving intravenous fluid was fixed at 2000 to 3000 cc. per day.

Intake in each case was calculated from the average of two or three analyses of duplicate diets and from the analysis of the saline solutions, when these were used. Output was determined by the analysis of 24-hour urine collections preserved with thymol and chloroform and from the analysis of stools pooled in three- to seven-day periods.

Urine and nitric acid digests of homogenized stools and diets were analyzed for sodium and potassium using an internal standard flame photometer. Determinations of chloride,[21] nitrogen,[1] phosphorus,[6] titratable acid,[13] ammonia,[7] and pH (glass electrode) were made on every urine specimen. All urines were acid and no attempt was made to prevent loss of carbon dioxide from the urine as it was collected. Each morning the fasting subjects were weighed on a beam-type balance to the nearest 10 grams. Every few days a sample of venous blood was withdrawn without stasis in an oiled syringe and determinations made of the serum concentrations of sodium and potassium, chloride,[21] and total carbon dioxide content.[20] In some cases whole blood pH was measured with the glass electrode and in the others serum pH was measured colorimetrically.[11]

The daily balance was calculated as net intake minus combined output in stool and urine. No liquid or unusually bulky stools were passed by any of the subjects in this study and daily fecal losses were assumed to be essentially constant within each collection period. The quantity of each substance determined in the pooled stool collections was therefore divided by the number of days in the collection period to obtain an average daily output. Changes in the volume of the "chloride space" and shifts of sodium and potassium into or out of intracellular water were calculated in the usual manner[18] on the assumption that "chloride space" is essentially equivalent to the volume of extracellular fluid and that the latter constituted 20% of the body weight at the beginning of each study.

Results

1. *General effects.* In all three of the subjects on high salt intake and in two of the three subjects on moderate salt intake, administration of DOCA produced an initial increase in weight which was most rapid in the high salt group. When treatment was continued for more than a week, the curve of weight gain tended to level off or to decline. A few subjects receiving salt developed mild peripheral edema. Two who were on high salt intakes showed occasional slight elevation of arterial diastolic pressure but there were no significant blood pressure changes in any of the others. Beginning several days after the initiation of treatment the majority of subjects complained of one or more symptoms including malaise, headache, anorexia, insomnia, and muscle cramps. In some cases these symptoms were severe enough to interfere with the optimal performance of daily tasks.

2. *Effects on urine electrolytes, nitrogen, and acidity.* The detailed balance data obtained from subject M. S., on a low salt intake, subject R. R., on a moderate salt intake, and subject B. Z., on a high salt intake, are presented in Tables 1, 2, and 3 respectively. The results obtained in each of these experiments are typical of those obtained for each of the three dietary groups.

M.S. ♀ 24

TABLE 1

Day	Medications	Body weight	Intake					Urine									Stool				Serum				
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl
								mEq./	mEq./	mEq./	mM./	mEq./	mEq./	gms./		mEq./	mEq./	mEq./	gms./		mEq./	mEq./	mEq./	mEq./
		kg.	cc.	mEq.	mEq.	mEq.	gms.	cc.	day	day	day	day	day	day	day		day	day	day	day		l.	l.	l.	l.
1		62.72	2110	14	64	15	12.2	2007	21	60	23	24	42	26	15.7	6.02	.1	2.0	.2	0.2		26	139	4.8	102
2		62.43	2110	14	64	15	12.2	1982	6	62	11	24	40	27	14.1	5.93	.1	2.0	.2	0.2					
3		62.42	2110	14	64	15	12.2	1897	4	53	11	24	45	27	13.5	5.59	.1	2.0	.2	0.2	7.31	27.5	139	4.5	102
4		62.39	2110	14	64	15	12.2	1822	4	60	8	25	43	26	12.9	5.87	.1	2.0	.2	0.2					
5	DOCA 20 mg.	62.11	2110	14	64	15	12.2	1905	2	55	7	23	43	28	14.1	5.77	.1	2.6	.3	0.3		27.5	139	4.5	103
6	DOCA 20 mg.	61.86	2110	14	64	15	12.2	1900	2	58	6	23	40	26	12.9	5.82	.1	2.6	.3	0.3					
7	DOCA 20 mg.	61.64	2110	14	64	15	12.2	1920	2	53	4	24	45	26	13.6	5.62	.1	2.6	.3	0.3	7.36	28.5	139	4.2	102
8	DOCA 20 mg.	61.62	2110	14	64	15	12.2	2175	2	64	7	25	40	21	13.5	5.89	.1	2.6	.3	0.3					
9	DOCA 20 mg.	61.45	2110	14	64	15	12.2	1965	2	58	5	22	40	19	13.8	5.90	.1	2.6	.3	0.3					
10	DOCA 20 mg.	61.60	2110	14	64	15	12.2	1965	2	46	4	22	43	24	13.4	5.87	.1	2.6	.3	0.3	7.33	29.0	137	4.2	103
11	DOCA 20 mg.	61.49	2110	14	64	15	12.2	1870	1	51	4	19	35	22	12.3	5.97	.1	2.6	.3	0.3					
12	DOCA 20 mg.	61.58	2110	14	64	15	12.2	1755	1	53	5	20	36	22	12.1	5.92	.1	2.6	.3	0.3	7.33	29.0	137	4.0	102
13	DOCA 20 mg.	61.49	2110	14	64	15	12.2	1555	1	54	5	20	35	21	11.8	5.88	.1	2.6	.3	0.3					
14	DOCA 20 mg.	61.57	2110	14	64	15	12.2	1522	1	50	3	20	36	22	12.9	5.66	.1	2.6	.3	0.3	7.37		137	4.3	104
15	DOCA 20 mg.	61.47	2110	14	64	15	12.2	1450	1	51	4	19	32	20	11.7	5.99	.1	2.6	.3	0.3					
16		61.22																			7.36	29.5	137	4.3	104

TABLE 2

Day	Medications	Body weight	Intake					Urine									Stool				Serum				
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl
									mEq./	mEq./	mEq./	mM./	mEq./	mEq./	gms./										
		kg.	cc.	mEq.	mEq.	mEq.	gms.	cc.	day	day	day	day	day	day	day		mEq./ day	mEq./ day	mEq./ day	gms./ day		mEq./ l.	mEq./ l.	mEq./ l.	mEq./ l.
1		68.84	3000	214	61	219	12.5	1860	108	71	131	28	57	32	14.5	6.3	.3	6.1	.3	0.8	7.40	32.0			
2		69.48	3000	214	61	219	12.5	2770	159	68	177	26	50	28	13.6	6.4	.3	6.1	.3	0.8	7.39	29.5	145	3.7	99
3		69.20	3000	214	61	219	12.5	2910	275	52	256	27	48	22	15.1	6.6	.3	6.1	.3	0.8	7.38	27.5	143	3.9	100
4		68.48	3000	214	61	219	12.5	2640	199	50	194	27	51	22	14.3	6.7	.3	6.1	.3	0.8					
5		68.30	3000	214	61	219	12.5	3120	255	56	257	29	52	30	13.9	6.1	.3	6.1	.3	0.8	7.42	29.5			102
6	DOCA 20 mg.	67.79	3000	214	61	219	12.5	3065	150	82	182	28	47	27	13.9	6.45	.2	6.7	.5	0.7					
7	DOCA 20 mg.	67.26	3000	214	61	219	12.5	2350	91	89	120	33	59	34	16.0	6.4	.2	6.7	.5	0.7	7.37	29.5	141	3.8	101
8	DOCA 20 mg.	68.00	3000	214	61	219	12.5	2900	144	89	179	27	56	24	14.1	6.5	.2	6.7	.5	0.7					
9	DOCA 20 mg.	68.00	3000	214	61	219	12.5	2980	142	77	162	27	53	22	13.3	6.6	.2	6.7	.5	0.7	7.40	29.5	142	3.3	101
10	DOCA 20 mg.	67.98	3000	214	61	219	12.5	2900	217	93	248	34	60	21	14.6	6.05	.2	6.7	.5	0.7					
11	DOCA 20 mg.	67.69	3000	214	61	219	12.5	2580	148	77	163	32	54	22	13.5	6.45	.2	6.7	.5	0.7	7.40	30.0	143	3.2	102
12	DOCA 30 mg.	68.05	3000	214	61	219	12.5	3100	222	70	247	25	51	13	10.7	6.65	.2	6.7	.5	0.7			143	3.0	
13	DOCA 30 mg.	67.54	3000	214	61	219	12.5	3770	292	97	307	35	71	23	15.6	6.7	.9	11.6	.5	1.2					
14	DOCA 30 mg.	66.35	3000	214	61	219	12.5	3000	162	72	193	33	75	29	13.7	65	.9	11.6	.5	1.2	7.37	31.0	143	3.0	100
15		66.20	3000	214	61	219	12.5	2490	183	30	146	24	56	15	13.6	6.8	.9	11.6	.5	1.2	7.40	32.0	143	2.8	100
16		66.29	3000	214	61	219	12.5	2770	236	15	180	19	37	9	11.4	6.9	.9	11.6	.5	1.2					
17		66.35	3000	214	61	219	12.5	2270	183	17	177	18	50	17	11.1	6.55	.9	11.6	.5	1.2	7.42	27.5	144	3.5	103
18		66.36	3000	214	61	219	12.5	3700	344	24	342	29	69	21	7.4	6.45	.9	11.6	.5	1.2					
19		65.44																							

In patient M. S., on low salt intake, (Table 1), administration of DOCA for 11 days produced no significant change in the renal excretion of any of the measured electrolytes. In the other two patients (Tables 2 and 3) there was a prompt reduction in excretion of sodium and chloride with a concomitant urinary loss of potassium. In these cases and in all the other subjects on the moderate and high salt intakes, the reduction in sodium excretion exceeded the decrease in chloride. Sodium retention was initially much greater than the urinary potassium loss, and tended to diminish progressively. Usually within a week after treatment with DOCA had begun, urinary sodium and chloride excretion were equal to, or greater than, the control levels, although the potassium diuresis persisted. A more detailed description and analysis of the changes in sodium, chloride, and potassium balance will be presented in Section 5. It is worth noting here, however, that in none of the three subjects with moderate salt intakes and normal urine volumes did treatment with DOCA reduce urine sodium concentration to the levels often observed during physiological states demanding sodium retention. The minimum urine sodium concentration during the administration of DOCA was 24 to 50 mEq./L.

In subjects R. R. (Table 2), B. Z. (Table 3), and in all the others receiving added salt, there was a small but definite rise in daily ammonia excretion which appeared within a few days after treatment had been started. The mean increment in ammonia excretion averaged 8 mEq./day for the subjects on moderate salt intake and 14 mEq./day for those on high salt intake. The maximum increment in ammonia excretion above the largest control value was 13-30 mEq./day for the subjects on high salt intake, and 11-18 mEq./day for the subjects on moderate intake. In the three patients on medium salt intake in whom post-treatment observations were made, ammonia excretion promptly returned to normal. A compensatory "rebound" occurred in only one patient (R. R.). These changes were apparently independent of urine pH, which did not change significantly. In one subject on low salt intake, R. R., there was a small transient increase of ammonia excretion at the inception of treatment, but there were no changes in the other two subjects, V. B. and M. S.

Urinary excretion of nitrogen, phosphorus, and titratable acidity was essentially unaffected by administration of DOCA in all subjects.

3. *Stool composition.* The average daily fecal excretion of sodium, potassium, and chloride for each group of three subjects before, during, and after treatment with DOCA is summarized in Table 4. These data are difficult to interpret because of the limited number of collection periods and the small quantities of electrolyte involved. The data suggest, however, that treatment with DOCA reduced stool sodium content in the high salt group and increased stool potassium content in all subjects. No changes in chloride content were discernible. It is of interest to note that during the

B.Z. ♂ 27.

TABLE 3

Day	Medications	Body weight	Intake					Urine									Stool				Serum				
			H_2O	Na	K	Cl	N	Vol.	Na	K	Cl	P	NH_4	T.A.	N	pH	Na	K	Cl	N	pH	CO_2	Na	K	Cl
		kg.	cc.	mEq.	mEq.	mEq.	gms.	cc.	mEq./day	mEq./day	mEq./day	mM./day	mEq./day	mEq./day	gms./day		mEq./day	mEq./day	mEq./day	gms./day		mEq./l.	mEq./l.	mEq./l.	mEq./l.
1		73.75	4600	474	72	483	14.3	3510	439	64	425	28	50	21	12.6	6.3	2.3	4.8	.8	1.2					
2		73.55	4600	474	72	483	14.3	3755	463	51	467	21	55	14	13.1	6.2	2.3	4.8	.8	1.2	7.36	26.5	145	3.8	107
3		73.20	4600	474	72	483	14.3	4000	510	54	520	21	56	17	12.4	6.0	2.3	4.8	.8	1.2					
4		72.97	4300	498	72	507	14.3	3310	404	46	420	17	51	21	12.6	6.1	2.3	4.8	.8	1.2	7.37	25.5	143	3.8	107
5	DOCA 20 mg.	73.09	4600	477	72	486	14.3	3290	313	63	365	17	49	33	12.0	5.4	1.2	5.9	.9	1.1	7.40	26.5	145	3.8	106
6	DOCA 20 mg.	73.02	4600	481	72	490	14.3	2520	277	82	325	26	57	29	13.4	6.2	1.2	5.9	.9	1.1		27.5	144	3.3	106
7	DOCA 20 mg.	74.16	4534	472	72	481	14.3	3520	358	93	439	24	62	29	13.7	5.9	1.2	5.9	.9	1.1					
8	DOCA 20 mg.	74.60	4600	481	72	490	14.3	3380	345	101	418	25	60	25	14.5	6.2	1.2	5.9	.9	1.1	7.43	30.0	142	3.3	105
9	DOCA 20 mg.	75.15	4600	481	72	490	14.3	3670	414	88	498	23	63	25	13.6	6.1	1.2	5.9	.9	1.1	7.45	31.0	146	3.2	103
10	DOCA 20 mg.	75.30	4600	481	72	490	14.3	3990	460	92	517	28	68	24	14.0	6.3	1.2	5.9	.9	1.1					
11	DOCA 20 mg.	75.32	4600	470	72	479	14.3	2980	429	54	454	22	87	10	14.5	6.5	1.2	5.9	.9	1.1	7.39	32.0	143	2.9	104
12		76.01																			7.38	31.0	146	2.8	103

control periods, stool sodium content was lowest in the subjects on low-salt diets.

4. *Serum electrolytes.* Serum potassium concentration decreased in every subject given moderate or large amounts of salt but did not change

TABLE 4
CHANGES IN STOOL ELECTROLYTE

	Low Na intake	*Medium Na intake*	*High Na intake*
		Average stool sodium, mEq./day	
Control	0.1	0.5	1.9
DOCA	0.1	0.4	0.8
Post-treatment	0.2	0.9	..
		Average stool potassium, mEq./day	
Control	3.7	6.9	5.2
DOCA	4.5	8.2	7.5
Post-treatment	5.3	8.8	..
		Average stool chloride, mEq./day	
Control	0.5	0.4	0.8
DOCA	0.6	0.4	0.9
Post-treatment	0.3	0.5	..

TABLE 5
CHANGES IN SERUM POTASSIUM

	Diet		*Serum K mEq./L.*		
Subject	*mM. NaCl per day*	*Days of DOCA*	*Initial*	*End of DOCA*	*Maximum post-treatment*
A. L.	530	5	4.5	3.7	...
E. A.	535	3	4.5	3.4	...
B. Z.	480	7	3.8	2.8	...
R. R.	214	9	3.9	2.8	...
E. A.	191	9	4.2	3.6	5.0
A. M.	182	10	4.4	3.6	5.3
R. R.	13	8	3.7	3.7	4.0
V. B.	15	9	3.8	4.1	4.0
M. S.	14	11	4.5	4.3	4.3

in the other three. These changes are summarized in Table 5. The reduction in potassium concentration in the subjects receiving salt was from .6 to 1.1 mEq. per liter, with no significant difference between the high and medium salt groups. In two of the three patients who were adequately studied in the post-treatment period (all on medium salt intakes), serum

potassium rose above the highest control value. In all but one of the subjects there were no changes in CO_2 content or chloride concentration in the serum and in none did the blood pH or serum sodium vary significantly. In one of the subjects (B. Z., Table 3) on a high salt intake, DOCA administration was accompanied by a rise of 4 mEq./liter in carbon dioxide content and an equivalent reduction in chloride.

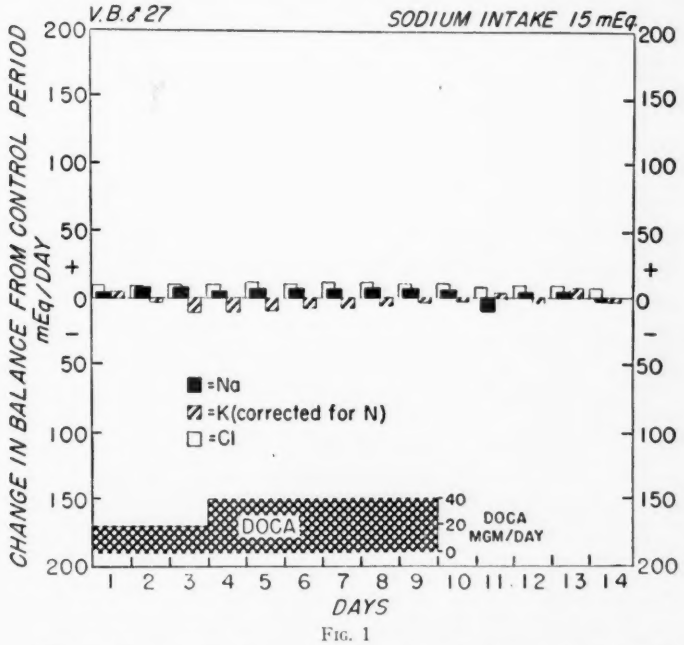

FIG. 1

Figures 1-9 show the changes in sodium, chloride, and potassium balances during and, in some instances, following DOCA administration. Values have been obtained by algebraically subtracting the average control daily balance from the daily balance during the treatment and post-treatment periods.

5. *Sodium chloride and potassium balances.* In Figures 1-9 are depicted the changes effected by DOCA in the net balances of sodium, chloride, and potassium (the latter was corrected for changes in nitrogen balance with an assumed K : N ratio of 2.7 mEq. K/gm. N). The data for these charts were obtained by algebraically subtracting the average control daily balance from the balance for each subsequent day. This can be assumed to correct for any systematic methodological error and for constant skin losses of electrolytes.

From Figures 1-3 it can clearly be seen that treatment of the three patients on a low-salt diet with 20 or 40 mgm. of DOCA per day for eight to eleven

days produced a small retention of sodium and chloride and only a small variable loss of potassium. In subject V. B. (Fig. 1) the potassium diuresis tended to decrease with continued treatment. In subject R. R. (Fig. 2) there was no change in potassium balance after three days of treatment with 20 mgm. of DOCA/day, but on the fourth and fifth days after the dose was

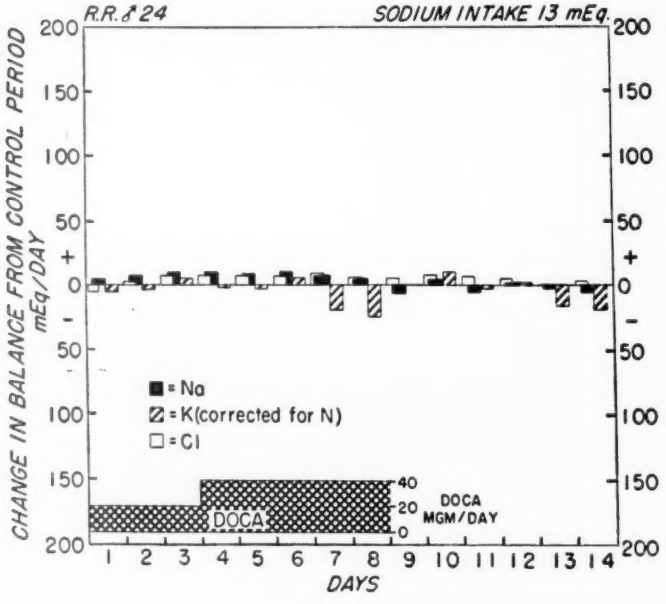

FIG. 2

doubled, small but probably significant potassium losses occurred. In neither of these latter two subjects was there any significant retention of potassium or loss of sodium in the post-treatment period.

In sharp contrast with the above results were the large changes in electrolyte balance observed in the six subjects given salt. In Figures 4-9 it may be seen that in each case DOCA produced an immediate retention of sodium and chloride and a loss of potassium. The large initial retentions of sodium and chloride quickly diminished, however, and usually by the end of a week daily sodium and chloride balance had returned to control levels or were actually negative. The total amount of sodium and chloride retention defined by these charts appeared to be proportional to the sodium intake, since the largest positive balance changes were seen in the subjects given large amounts of salt (Figs. 7-9).

By contrast, the loss of potassium in excess of nitrogen continued unabated in most cases for as long as the hormone was given. In subjects E. A. (Fig. 4) and B. Z. (Fig. 7), however, the potassium balance became reversed on the ninth and seventh days of treatment, respectively.

TABLE 6

CHANGES IN POTASSIUM BALANCE FROM CONTROL PERIOD (CORRECTED FOR NITROGEN)

Subject	*Days*	*Total for period mEq.*	*Average daily mEq.*
High salt intake			
A. L.	5	—92	—18
E. A.	3	—132	—44
B. Z.	6	—191	—32
Moderate salt intake			
R. R.	9	—237	—26
E. A.	8	—172	—22
A. M.	10	—185	—19

TABLE 7

INTERNAL BALANCES

	Salt intake mM. per	*Control*			*DOCA*			*Post-treatment*		
Subject	*day*	*Days*	*ICNa*	*ICK*	*Days*	*ICNa*	*ICK*	*Days*	*ICNa*	*ICK*
A. L.	530	2	+28	—8	5	+21	—133	..	.....	.....
E. A.	535	5	—61	—44	3	+86	—147	..	.....	.....
B. Z.	480	4	—59	+45	7	+145	—88	..	.....	.....
R. R.	214	5	+62	+10	9	+95	—205	4	—81	+94
E. A.	191	5	—189	—47	9	—90	—242	5	—46	+68
A. M.	182	12	—55	+101	10	+112	—114	10	—295	+237

In Table 6 are presented the cumulated and average daily potassium balances during administration of DOCA. These data are derived from Figures 4-9, but the final days in Figures 4 and 7, when potassium balances became positive again, have been omitted. The mean potassium losses averaged 27 mEq./day for the entire group. The daily losses appeared to be slightly greater in the subjects on high salt intake, but this difference is not of statistical significance. The mean total cumulated loss of potassium in excess of nitrogen was 168 mEq. Despite the magnitude of these changes, serum bicarbonate concentration changed in only one subject, and in this case only slightly.

In each of the three subjects on moderate salt intake in whom post-treatment periods were obtained (Figs. 4-6) cessation of treatment with DOCA resulted in a more or less prompt reversal of the initial changes in electrolyte balance.

6. *Internal balances of sodium and potassium.* In general, the changes in external sodium and chloride balance were parallel, but in all except one of

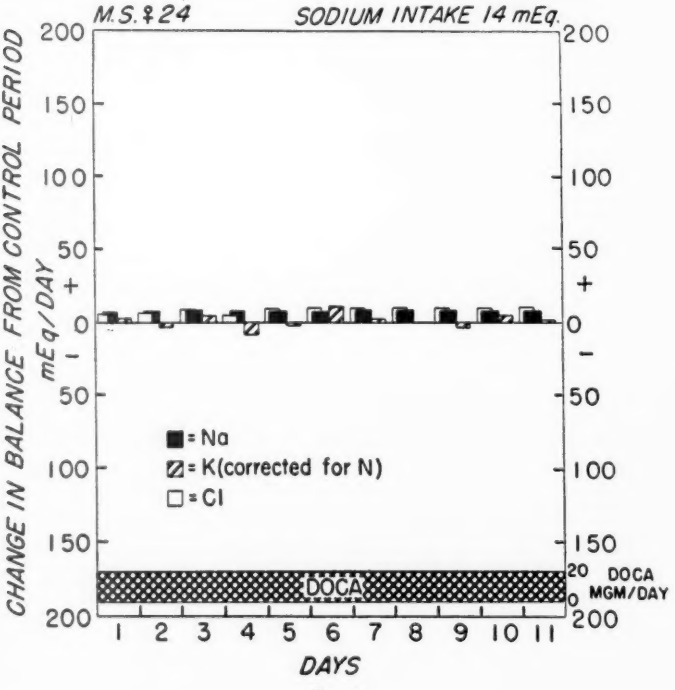

FIG. 3

the subjects receiving added salt (E. A., Fig. 4) the retained sodium exceeded the retention of chloride by more than the ratio of sodium to chloride in extracellular water. Serum concentrations of sodium and chloride were virtually unchanged. Therefore, assuming the extracellular position of chloride, this excessive retention of sodium defines a shift of sodium into intracellular water. On the other hand, the large losses of potassium in excess of nitrogen with only small changes in serum concentration must have resulted from a movement of potassium out of cells.

These changes in internal balance were calculated separately for the periods before, during, and after treatment with DOCA and are sum-

marized in Table 7. It should be noted that these calculations are based on the total cumulated balances for each period and do not refer to the changes in daily balance depicted in Figures 4-9. The tabulated data show that in five of the six subjects given salt, treatment with DOCA caused a shift of sodium into cells of from 21 to 145 mEq., and a simultaneous loss of cellular potassium of from 88 to 205 mEq. There was no apparent quantitative

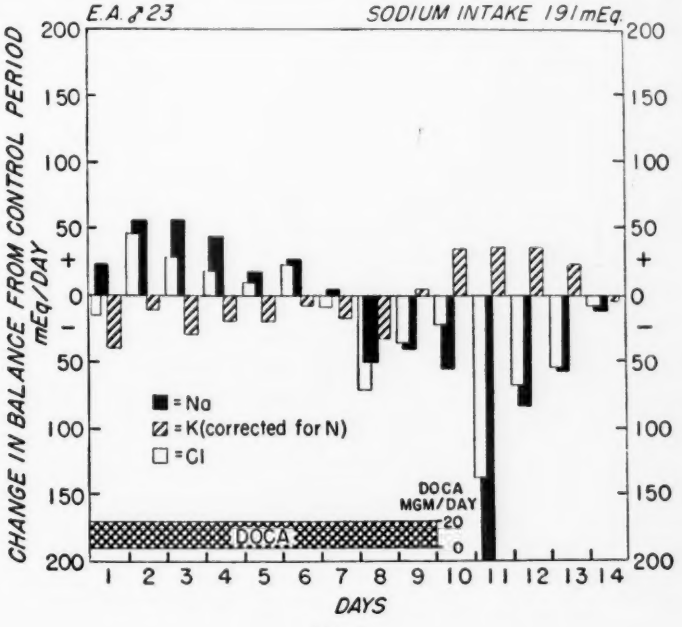

FIG. 4

correlation between the cellular exchanges of sodium and potassium during the treatment period. In the subjects on moderate salt intake followed through a post-treatment period, these changes became approximately reversed.

DISCUSSION

The data presented here afford ample confirmation of the fact that DOCA produces in normal subjects given sodium chloride an initial retention of sodium and chloride and an increased excretion of potassium without change in nitrogen or phosphorus balance. It seems equally clear, however, that with the doses employed here the initial effect on sodium and chloride is only transient and usually disappears within a week despite

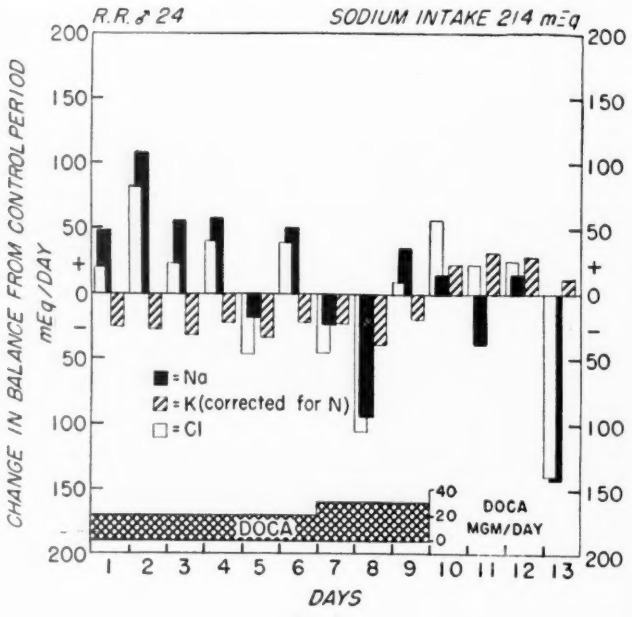
R.R. ♂ 24
SODIUM INTAKE 214 mEq
CHANGE IN BALANCE FROM CONTROL PERIOD
mEq/DAY
■ = Na
▨ = K(corrected for N)
□ = Cl
DOCA
40
20
0
DOCA MGM/DAY
DAYS

FIG. 5

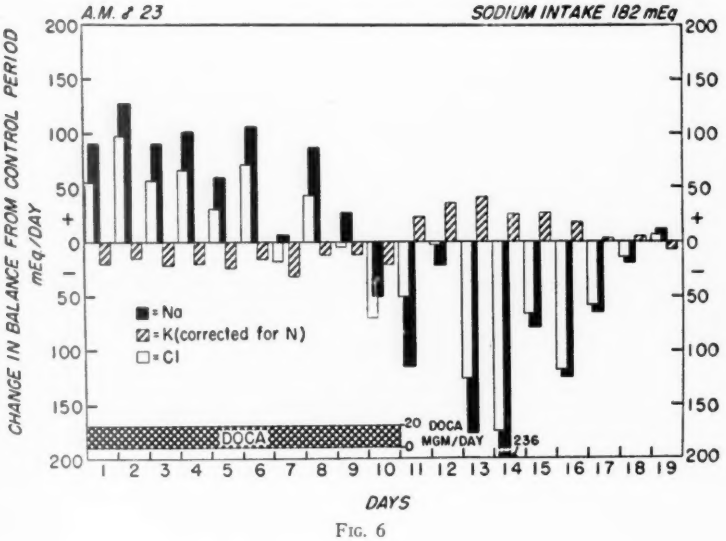
A.M. ♂ 23
SODIUM INTAKE 182 mEq
CHANGE IN BALANCE FROM CONTROL PERIOD
mEq/DAY
■ = Na
▨ = K(corrected for N)
□ = Cl
DOCA
20
0
DOCA MGM/DAY
236
DAYS

FIG. 6

continued treatment. The potassium diuresis is at first unaffected by the restoration of sodium balance, but in two of our subjects potassium balance was restored to normal on the seventh and ninth days of treatment respec-

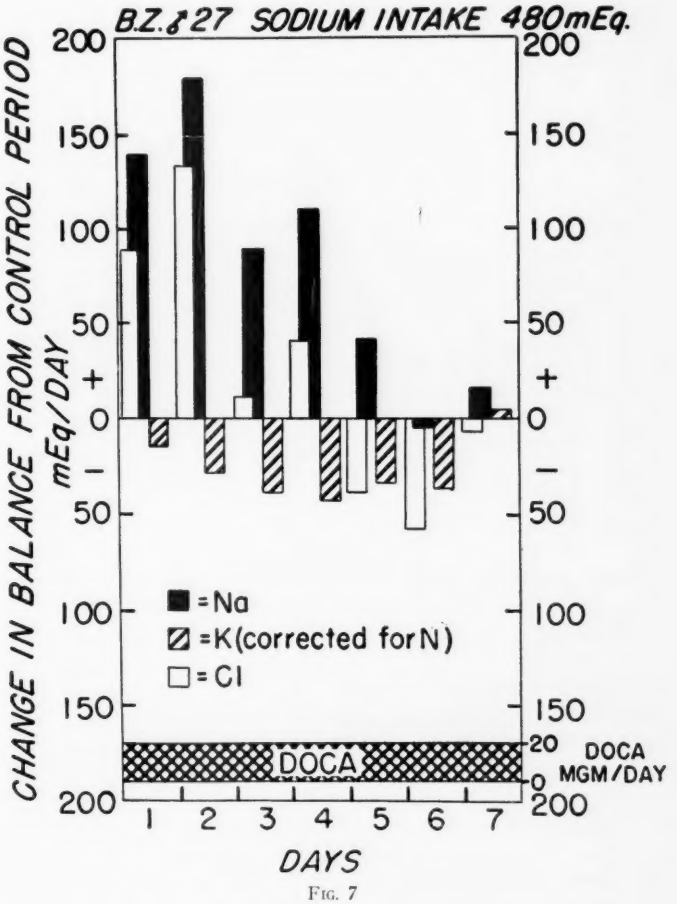

FIG. 7

tively. Similar observations have been made with regard to the transitory influence of DOCA on sodium balance[4, 22] and on sodium and potassium balance.[19] In this respect the action of DOCA on electrolyte balance resembles that of Cortisone[27] and Compound F.[24]

Escape of sodium balance from the initial effects of Cortisone or Compound F might be attributed to induced adrenocortical insufficiency[27]

but there is evidence that treatment of normal men with DOCA in doses comparable to those used here does not inhibit endogenous adrenal steroid production.[6] The absence of any post-treatment sodium diuresis or potassium retention in patients V. B. (Fig. 1) and R. R. (Fig. 2), who were not given salt during the period of study, as well as the persistence of reduced sweat sodium content even after renal "escape" has occurred[8] also argue against the possibility of adrenal inhibition with DOCA. Rapid develop-

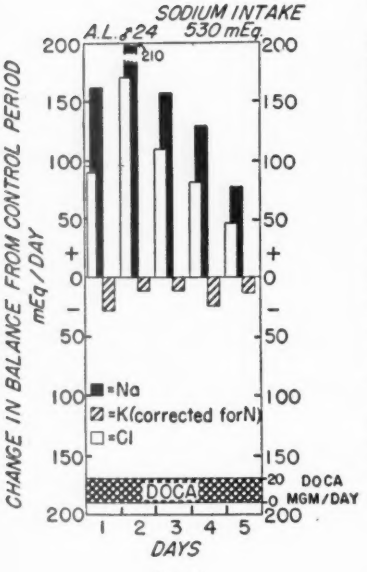

FIG. 8

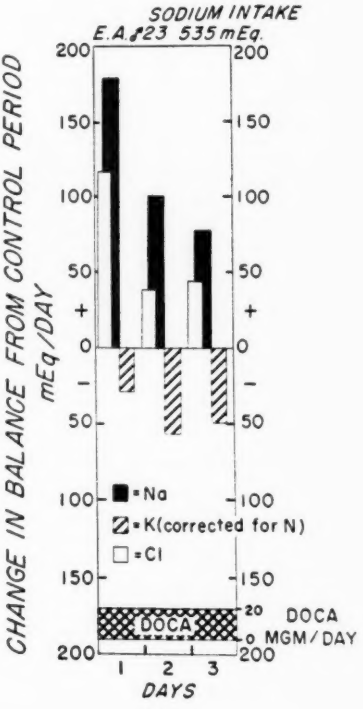

FIG. 9

ment of relative refractoriness to the effects of DOCA by renal tubular cells is a possibility which cannot be evaluated at present, but it must be considered unlikely in view of the fact that DOCA can maintain sodium balance in patients with adrenocortical insufficiency for indefinite periods. It seems more probable that sodium balance was restored by other regulatory mechanisms. These might involve rapid expansion of plasma volume and increase in glomerular filtration rate, changes which usually occur following treatment of normal subjects[5, 14] or dogs[9] with salt-retaining adrenal

steroids and which are often associated under other circumstances with increased salt excretion.[21, 26]

The dependence of potassium diuresis upon the level of sodium chloride intake was one of the striking findings in this study. In one subject on a low-salt diet (M. S., Table 1 and Fig. 3) there was absolutely no loss of potassium. This phenomenon has been independently observed by Seldin, Welt, and Cort.[25] In the other two subjects on low-salt diets the losses of potassium were much smaller and less sustained than those observed in all of the six other subjects given added salt. Thus it would appear that the potassium diuresis produced by DOCA is dependent in some way upon sodium chloride intake. Similar conclusions may be drawn from observations on the effects of Compound F.[24]

The continuation of potassium loss for several days after sodium retention had stopped indicates that there is no direct relationship between net retention of sodium and loss of potassium. It is possible, however, that increased potassium excretion depends upon an augmented rate of renal tubular reabsorption of sodium, which continues while DOCA is administered, even after increases in glomerular filtration rate have restored sodium excretion to normal. The smaller, more variable increases in potassium excretion observed in two of the subjects on the low-salt diet may have resulted from the smaller increments in sodium reabsorption permitted under these circumstances. The eventual dissipation of potassium diuresis may be the result of hypokalemia and a diminishing load of potassium delivered to the renal tubules, or else the direct result of renal tubular potassium depletion.

It is of interest to note that relatively large losses of potassium in excess of nitrogen occurred in our subjects without the development of alkalosis. Others have reported that alkalosis may accompany potassium deficits of this magnitude produced by administration of ACTH or Cortisone.[27] This difference cannot be attributed to the relation of dietary chloride to sodium[20] because this was the same in both groups. It would appear either that the difference is due to chance variation in the individual response of the subjects studied or that factors other than potassium depletion are involved in the production of alkalosis with ACTH and Cortisone.

The internal balances summarized in Table 6 show no quantitative relationship between exchanges of sodium and potassium. However, these data must be interpreted with the greatest caution because in only a few instances are the calculated shifts large enough to be of even statistical significance.[28] The direction of the shifts is nevertheless uniform enough to allow the conclusion that during treatment with DOCA cell balances of sodium probably did become positive while corrected potassium balances became negative. It is also worth noting that no significant cellular shifts occurred during the control period in the subjects given large quantities of extra salt as daily saline infusions. Other workers have noted that large

loads of sodium chloride did not increase intracellular sodium content in normal adults[17, 28] or in normal dogs[18] but intracellular Na content was apparently increased by sodium chloride loading in a normal infant studied by Gamble *et al.*[8]

The changes produced in renal ammonia secretion are of considerable significance although they were small and variable. There was no relation between urine acidity and ammonia content. It is of interest, moreover, that ammonia excretion increased in all of the subjects given salt although it was affected, and then but slightly, in only one of the three subjects on a salt-free diet. Others have shown that adrenal insufficiency reduces renal synthesis of ammonia both *in vivo*[10, 15] and *in vitro*[15] and that administration of DOCA or added dietary salt will improve this function in adrenalectomized dogs.[10] Interpretation of these observations must await further study of the factors controlling ammonia production.

Summary and Conclusions

Electrolyte and nitrogen balance studies have been carried out on normal young adults who were given 20 to 40 mgm. of DOCA intramuscularly for periods of three to eleven days.

In six subjects whose diets were supplemented with moderate or large amounts of salt, administration of DOCA produced immediate retention of sodium and chloride, and a loss of potassium. The retention of sodium exceeded that of chloride and was greatest in subjects given the highest salt intake. With continued administration of the hormone, sodium retention diminished, and often a sodium diuresis appeared.

The negative balance of potassium averaged 27 mEq./day. This loss, unaccompanied by change in nitrogen balance, continued beyond the period of sodium retention, and regularly resulted in hypokalemia. The cumulative deficits of potassium ranged from 92 to 237 mEq., and were accompanied by intracellular shifts of sodium. There were no significant changes in serum bicarbonate or chloride concentration.

The three subjects on low sodium diets failed to show the changes in potassium balance described above. In one of these subjects there was no loss of potassium; in another, potassium diuresis did not appear until the seventh day of treatment; in the third subject, loss of potassium was minimal and only transient.

Ammonia excretion increased in all the subjects given salt but rose in only one subject on a low-salt diet. In the latter instance, the change was small and transitory. In no subjects were there significant changes in urine pH, titratable acidity, or in phosphorus excretion.

The observations in this study are consistent with the view that the increased potassium excretion as well as the small increases in ammonia secretion produced by treatment with DOCA are in some way conditioned by the simultaneous augmentation of renal tubular reabsorption of sodium

and chloride. Eventual escape from the sodium-retaining effect of DOCA is probably the result of regulatory mechanisms which override the hormonal effect on the renal tubule.

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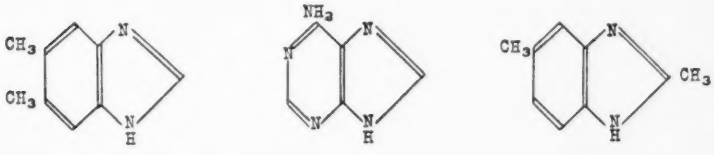
INHIBITION OF INFLUENZA VIRUS MULTIPLICATION BY 2,5-DIMETHYLBENZIMIDAZOLE*

IGOR TAMM,† KARL FOLKERS, AND FRANK L. HORSFALL, JR.

It can be postulated that the striking biological specificity shown by viruses is determined by or associated with certain specific nucleoproteins contained in the viral particle. If this be true, then it might be possible to inhibit viral multiplication without interfering with the function of host cell nucleoproteins, provided that sufficiently selective means were available. Such means are not available as yet and exploration of the nucleoprotein metabolism of virus infected host cells appears important. Studies of this kind have been undertaken with bacterial cells infected with bacterial viruses[5–8, 14, 15] but it has not been feasible to conduct similar investigations on infected animal host cells. Such studies should result in the acquisition of knowledge which might indicate new approaches to the problem of selective viral inhibition and increase understanding of the nature and mechanisms of the biological specificity exhibited by viruses.

The use of benzimidazole derivatives in such an investigation appeared fruitful. Vitamin B_{12} is known[3] to contain a benzimidazole moiety, i.e., 5,6-dimethylbenzimidazole (I).[2–4, 12]



I II III

The recent observations of several groups of investigators that thymidine can replace vitamin B_{12} in the nutrition of several lactic acid bacteria[17] were quickly extended, and it was learned that the desoxyribosides of adenine, guanine, hypoxanthine, and cytosine are as effective as thymidine.[17] Desoxyribonucleic acid can also be utilized slowly in place of vitamin B_{12} by some of these microorganisms.[13] A close metabolic relationship between desoxyribonucleic acid and vitamin B_{12} is apparent from this evidence which involves microbial growth. Organic chemical evidence supporting this rela-

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tionship is provided by the recent studies on the hydrolysis of pseudovitamin B_{12}. The occurrence of adenine (II) instead of 5,6-dimethylbenzimidazole in the molecule has been demonstrated.[9]

The structure of the benzimidazole (I) in vitamin B_{12}, and the purine (II) have the same bicyclic skeleton with respect to size and a common imidazole ring, and it is apparent that analogs of the benzimidazole are of considerable interest as regards possible selective inhibitory activity on viral multiplication. The initial studies of 5,6-dimethylbenzimidazole and related derivatives for growth-promoting and growth-inhibitory effects in rats revealed that 2,5-dimethylbenzimidazole (III) appeared to be unique, in preliminary tests, by causing a growth depression.[10] This substance, therefore, was particularly suitable for beginning a study of the effects of benzimidazole derivatives on viral multiplication.

In this report the inhibition of influenza B virus (Lee strain) multiplication by 2,5-dimethylbenzimidazole is described in some detail. The effect of this compound on the multiplication of influenza A virus (PR8 strain) also is described.

Materials and Methods

Chorioallantoic membranes. Chorioallantoic membranes from 10-15-day-old chick embryos were removed, washed with nutrient fluid (see below), and cut into pieces of suitable size. The average weight of individual membranes was 250 mgm. For different experiments, 2, 4, or 6 pieces were cut from each membrane and one piece was suspended in each tube which contained 2 ml. of nutrient fluid; 25 x 150 mm. tubes were used and were closed with rubber stoppers (No. 4). The membranes were incubated at 35° C. and shaken horizontally at a rate of 90 strokes per minute with a stroke length of 9 cm.

Composition of nutrient fluid. A modified glucosol solution was prepared according to Fulton and Armitage.[11] This had the following composition: NaCl, 8 gm.; $CaCl_2$, 0.2 gm.; $MgCl_2 \cdot 6H_2O$, 0.5 gm.; glucose, 1.0 gm.; glass distilled H_2O to 1000 ml.

Depending on the pH desired, M/15 phosphate buffer was prepared in the following ways:

1. Na_2HPO_4	4.736 gm.	
KH_2PO_4	4.539 gm.	pH 6.8
Glass distilled H_2O to	1000 ml.	
2. Na_2HPO_4	7.105 gm.	
KH_2PO_4	2.269 gm.	
Glass distilled H_2O to	1000 ml.	pH 7.28

These solutions were autoclaved separately. Equal volumes of modified glucosol and phosphate buffer, either 1 or 2, were mixed. These were designated nutrient fluid (N.F.) 1 and 2, respectively. Penicillin and streptomycin were added to a final concentration of 10 units and 40 μg. per ml., respectively. The N.F. also contained phenol red, 20 mgm. per liter, as a pH indicator.

When necessary in the case of solutions containing supplements which altered the pH of the solution, the pH was readjusted with N/10 NaOH or HCl. During incubation, the pH of the N.F. usually was maintained within 0.1 of a pH unit.

Viruses. Lee and PR8 strains of influenza B and A viruses were used. Each virus was inoculated into the allantoic sac of 10- or 11-day chick embryos. The Lee infected

eggs were incubated at 35° C. for 32 hours, whereas the PR8 infected eggs were incubated at the same temperature for 24 hours. These periods of incubation, which are shorter than those usually employed, were chosen in order to assure maximal quantities of infectious and minimal quantities of non-infectious virus in the allantoic fluid. The eggs were then chilled at —28° C. for one hour. After harvest, the allantoic fluid was stored in 1 ml. amounts at —65° C. Each tube of seed virus was used but once. The hemagglutination titer was 1:4096 and the infectivity titer was $10^{-6.5}$ for both viruses.

Red blood cells. Blood from individual chickens was collected into ACD[16] solution and stored at 4° C. The red cells were washed three times with 0.85% NaCl buffered at pH 7.2 with 0.01 M phosphate, and a 0.5% suspension by volume was prepared.

Hemagglutination titrations. Serial twofold dilutions of infected allantoic or nutrient fluid were made in 0.5 ml. volumes of buffered saline in round bottom Kahn tubes and 0.5 ml. of RBC suspension was added to each tube (final concentration = 0.25%). Tests were read after one hour at 25° C. The last tube showing strong agglutination (2 to 3+) was considered as the end point. The titer was expressed as dilution at the end point. Each titer recorded in the tables represents the geometric mean of the individual titers obtained on the fluids from a group of 4-6 membrane preparations used with each variable. In several experiments, infectivity titers were also determined and good correspondence between hemagglutination and infectivity titers was found.

Infectivity titrations. Serial tenfold dilutions of virus were made in buffered saline in an icebath and dilutions were inoculated allantoically into groups of 10-day-old chick embryos, 0.1 ml. per egg. Eggs were incubated at 35° C. for 48 hours. After chilling overnight at 4° C. or for one hour at —28° C., the allantoic fluids were harvested and tested for hemagglutinating ability. The 50% embryo infectivity end point ($E.I.D._{50}$) was calculated in the usual manner.

Experimental Results

Inhibition of Lee Virus Multiplication by 2,5-Dimethylbenzimidazole

Effect of concentration of 2,5-dimethylbenzimidazole. Portions of chorioallantoic membrane from 11-day-old embryonated eggs were suspended in N.F.1. The average wet weight of the portions of membrane used was 130 mgm. The concentration of 2,5-dimethylbenzimidazole was 0.0017, 0.0025, or 0.0034 M. The concentration of Lee virus added was $10^{6.5}$ $E.I.D._{50}$ per ml. The membrane was added to N.F. already containing both the compound and the virus. Control tubes contained no compound. After incubation for 41 and for 65 hours, the concentration of virus was measured by the hemagglutination procedure.

Table 1 summarizes the results of such an experiment. As can be seen, the degree of inhibition was markedly dependent on the concentration of 2,5-dimethylbenzimidazole.

Variation in the concentration of the compound by a factor of 1.4 resulted in a change in the extent of viral inhibition by a factor of 4 or greater. The extent of inhibition was closely comparable at 41 and at 65 hours of incubation.

Effect of concentration of virus. Portions of chorioallantoic membrane from 13-day-old embryonated eggs were suspended in N.F.1 containing

2,5-dimethylbenzimidazole in a concentration of 0.0025 M. The portions of membrane weighed 130 mgm. The concentration of Lee virus added was $10^{6.5}$, $10^{5.5}$ or $10^{4.5}$ E.I.D.$_{50}$ per ml. The membrane was added to N.F. already containing all the other components. Hemagglutination titrations were carried out at 23 and 48 hours.

TABLE 1

EFFECT OF CONCENTRATION OF 2,5-DIMETHYLBENZIMIDAZOLE ON INHIBITION OF LEE VIRUS MULTIPLICATION

2,5-Dimethyl-benzimidazole	*Hours of incubation*			
	41		*65*	
M/liter	*Hemagglut. titer*	*% of control*	*Hemagglut. titer*	*% of control*
None	350*	100	750	100
0.0017	160	46	260	35
0.0025	45	13	64	9
0.0034	3	1	11	1

* Expressed as the reciprocal.

TABLE 2

EFFECT OF SIZE OF INOCULUM ON INHIBITION OF LEE VIRUS MULTIPLICATION BY 2,5-DIMETHYLBENZIMIDAZOLE

Viral inoculum		*Hours of incubation*			
		23		*48*	
log E.I.D.$_{50}$		*Hemagglut. titer*	*% of control*	*Hemagglut. titer*	*% of control*
6.5	C*	110	100	440	100
"	2,5-D†	20	18	110	25
5.5	C	20	100	180	100
"	2,5-D.	<1	<5	4	2
4.5	C	<1	...	270	100
"	2,5-D.	<1	...	<1	<1

* C = Control containing no compound.
† 2,5-D. = 2,5-Dimethylbenzimidazole, 0.0025 M.

Table 2 summarizes the results of an experiment of this kind. It can be seen that the degree of inhibition was much more marked as less virus was used in the inoculum. At 23 hours, the titers of control specimens which had received smaller inocula were considerably lower than the specimen which contained $10^{6.5}$ E.I.D.$_{50}$. At 48 hours, the concentration of virus in all the control specimens had reached similar high levels.

Effect of order of mixing. Portions of chorioallantoic membrane from 11-day embryonated eggs were suspended in 1 ml. of N.F.2 containing sufficient Lee virus to yield a final concentration of $10^{5.5}$ E.I.D_{50}. The portions of membrane weighed 130 mgm. After incubation for one hour, 1 ml. of N.F.2 containing 2,5-dimethylbenzimidazole to give a final concentration of 0.0025 M was added per tube. At 31 hours and 49 hours, hemagglutination titers were determined.

TABLE 3

INHIBITION OF LEE VIRUS MULTIPLICATION BY 2,5-DIMETHYLBENZIMIDAZOLE ADDED ONE HOUR AFTER VIRUS

		Hours of incubation			
		31		*49*	
Viral inoculum		*Hemagglut. titer*	*% of control*	*Hemagglut. titer*	*% of control*
log E.I.D_{50}					
5.5	C.	180	100	304	100
"	2,5-D.*	6	3	18	6

* 0.0025 M.

TABLE 4

EFFECT OF 2,5-DIMETHYLBENZIMIDAZOLE ON THE CAPACITY OF CHORIOALLANTOIC MEMBRANE TO SUPPORT LEE VIRUS MULTIPLICATION

			Hours of incubation			
			42		*68*	
	2,5-Dimethylbenzimidazole		*Hemagglut. titer*	*% of control*	*Hemagglut. titer*	*% of control*
Group	*Conc.*	*Time*				
1	None	0-68	49	100	100	100
2	0.0025	0-42	<1	<2	...	...
"	0.0006	43-68	...	...	95	95
3	0.0025	0-68	1	2	2	2

The results, summarized in Table 3, indicate that in this experiment multiplication of the virus was inhibited to approximately the same extent as in experiments in which the membrane was exposed simultaneously to 2,5-dimethylbenzimidazole and to the virus.

Mode of action of 2,5-dimethylbenzimidazole. The effect of 2,5-dimethylbenzimidazole on the capacity of the chorioallantoic membrane to support viral multiplication was studied in experiments in which, after a 24-48-hour period of incubation, the concentration of the compound was reduced to essentially non-inhibitory levels by dilution with nutrient fluid. The results

of a representative experiment are given in Table 4. In this experiment, 90 mgm. portions of membrane from 14-day eggs were suspended in N.F.2 (Group 1) or in 0.0025 M 2,5-dimethylbenzimidazole in the same diluent (Groups 2 and 3) and inoculated with sufficient Lee virus to give $10^{5.5}$ $E.I.D._{50}$ per ml. After incubation for 42 hours, hemagglutination titers were determined and to tubes in Groups 1 and 2 three volumes of N.F.2 were added, whereas Group 3 received 2,5-dimethylbenzimidazole in N.F. 2. At 68 hours, aliquots again were withdrawn and the concentration of virus was measured. As can be seen, the multiplication of Lee virus at 42 hours in the treated groups (2 and 3) was limited to less than 2% of the controls. At 68 hours, the same degree of inhibition was found in Group 3. However, in Group 2, in which the concentration of the compound had been reduced at 42 hours from 0.0025 M to 0.0006 M, the titer was the same as in the control, indicating that the treated membrane retained, in undiminished degree, the capacity to support viral multiplication.

Experiments were carried out to determine if 2,5-dimethylbenzimidazole had a direct inactivating effect on Lee virus. $10^{6.5}$ $E.I.D._{50}$ of virus per ml. of N.F.1 or N.F.2 was held in the presence of 0.0034 M of the substance for 2 hours at 35° C. Control tubes, containing virus but no compound, were handled similarly. Infectivity titrations then were carried out in embryonated eggs. In three experiments, no difference was found between the virus titers of the 2,5-dimethylbenzimidazole treated specimens and the controls.

Lack of effect of 2,5-dimethylbenzimidazole on adsorption of Lee virus by the chorioallantoic membrane was established in experiments conducted as follows: $10^{7.5}$ $E.I.D._{50}$ of virus per ml. of N.F.1 was absorbed with 400 mgm. of membrane from 12-day embryonated eggs in the presence or absence of 0.0034 M of the compound. The specimens were held for 30 minutes at 35° C. and then centrifuged at 1,500 r.p.m. for five minutes. Under these conditions, 75% of the virus, as determined by hemagglutination titrations, was adsorbed regardless of the presence or absence of 2,5-dimethylbenzimidazole.

Experiments designed to show if the substance had an effect on agglutination of RBC by Lee or PR8 virus indicated that in a concentration of 0.0034 M the compound did not inhibit agglutination of chicken RBC by these agents nor did the substance itself cause agglutination of such RBC.

Studies on the oxygen uptake of chorioallantoic membranes were conducted in the Warburg apparatus. They showed that 2,5-dimethylbenzimidazole, in the concentrations employed in this investigation, did not affect the O_2 consumption of uninfected or Lee virus infected tissue. Moreover, the O_2 consumption of virus infected tissue was not significantly different from that of normal membranes as was found also by another worker using PR8 virus.[4]

So far, all attempts to block the inhibitory action of 2,5-dimethylbenzimidazole by vitamin B_{12} have failed. In view of the fact that a 1,2-diamino-4,5-dimethylbenzenoid moiety is present in vitamin B_{12}, as well as in riboflavin, the latter also was used in blocking experiments. These experiments yielded negative results.

Inhibition of PR8 Virus Multiplication by 2,5-Dimethylbenzimidazole

In two experiments, 0.0017 M 2,5-dimethylbenzimidazole caused inhibition of PR8 virus multiplication to an extent comparable to the inhibition obtained with Lee virus. Because of the greater stability of Lee virus at 35° C. in the media employed, this agent was chosen for more detailed studies in order to assure greater accuracy in the measurement of viral concentration.

Discussion

Several facts indicate that 2,5-dimethylbenzimidazole inhibits the multiplication of influenza viruses through an effect upon the host cell rather than on the virus. The compound actively inhibits Lee virus multiplication even when given sufficiently long after inoculation of a large amount of virus to assure that the process of intracellular multiplication of the agent is well under way. The lack of any effect of the compound on the adsorption of the virus by the chorioallantoic membrane and the finding that the virus is not inactivated by the substance support this view. Moreover, the fact that the membrane maintains its capacity to support viral multiplication, despite prolonged exposure to the compound, clearly indicates that the inhibitory activity is not attributable to a nonspecific or irreversible effect upon the host cells. In addition, the lack of an effect of the compound on the O_2 consumption of the membrane suggests that metabolic pathways other than those concerned with oxidative reactions are affected.

The marked dependence of the degree of inhibition on the amount of virus inoculated merits comment. If it is assumed that inhibitory concentrations of 2,5-dimethylbenzimidazole limit the multiplication of Lee virus in infected cells to a fixed fraction of the control yield, then small inocula would be expected to show a greater degree of inhibition during a fixed time interval. With small inocula, more cell-cycles of multiplication are required before maximal titers are reached than is the case with large inocula. If the effect of the compound in limiting multiplication is operative during each successive cycle, then the number of cycles required before maximal titers are reached will be increased because the total yield from one cycle affects that from the next. In theory, maximal titers should ultimately be reached despite the presence of the substance. However, with sufficiently high concentrations of 2,5-dimethylbenzimidazole, the time required to attain this end becomes very long indeed. During this period it is probable

that other factors would come into play and alter the final result in favor of the host cell.

The mechanism by which 2,5-dimethylbenzimidazole achieves an inhibitory effect on the multiplication of influenza viruses has not been established as yet. It appears possible, however, that the use of purine and pyrimidine bases as well as nucleosides in attempts to block the inhibitory action of this compound may throw some light on this important problem.

Summary

2,5-Dimethylbenzimidazole inhibits the multiplication of influenza B virus (Lee strain) or influenza A virus (PR8 strain) in the chorioallantoic membrane *in vitro*. The compound is inhibitory when given one hour after inoculation of a large amount of virus. It does not inactivate, nor does it interfere with, adsorption of the virus by the membrane. In concentrations which inhibit Lee or PR8 virus multiplication, the compound does not decrease O_2 consumption of the membrane. Prolonged exposure of the membrane to the compound does not diminish the capacity of the membrane to support Lee virus multiplication.

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ADDRESSES DELIVERED AT THE MEMORIAL EXERCISES

for

FRANCIS GILMAN BLAKE

in the

Historical Library
Yale University School of Medicine

June 15, 1952

FRANCIS GILMAN BLAKE

I would speak of Francis Gilman Blake as the teacher and preceptor, but to do this one must see him against the background of the Yale Medical School as it appeared to a student in the twenties. These were the unhurried years when the faculty could concentrate on their immediate duties and studies, essentially free from outside responsibilities. The institution was in a revival under the banner of full-time medicine. The staff was young and enthusiastic, possessing the confidence derived from the assurance that they were building a new pattern of medical education. Under the leadership of Milton C. Winternitz the school was not to be merely a training school for practitioners but an educational institution for students of medicine. In the clinical years lectures and recitations were almost unknown; the number of patients was such that the student had ample time to study his patients and their disabilities. Elective courses and the thesis provided a medium through which the student could become familiar with, and participate in, the experimental approach.

Francis Blake belonged in that environment and was a major influence in its creation. At times it seemed to be an atmosphere to which he was born, but there had been other factors. A few years ago he told me the most valuable course he ever had was one given by Theobald Smith in which the student selected a problem and then just worked on it. This may have been the beginning of an opinion he expressed concerning the medical curriculum —that if you had the right kind of students and gave them enough time for thinking and learning, the arrangement of courses made little difference. Moreover, he was one of the company of brilliant young physicians who had become convinced, largely through the influence of the Hospital of the Rockefeller Institute, of the advantages of full-time medicine for the student and the staff. They were the chosen because they had chosen.

It is doubtful whether a man in any other calling can mold the thoughts and the standards of professional endeavor of his students so completely and so enduringly as in that of the teacher-physician. As a teacher of medical science he can direct the synthesis of the diversified reactions learned in the impersonal laboratories of the basic sciences into a metabolic pool from which to draw understanding of the abnormalities of sick humanity. As a clinician he can demonstrate by direct example the manner in which scientific correlations, sometimes developed over centuries of observation, can be fitted like elements into the diagnosis of a case. He must then accept the responsibility and undertake with calm sympathy and honest judgment the care of what is no longer a case identified by clinical brilliance, but a troubled human creature. Finally, he can more frequently than not point out

that the amazing array of knowledge is far from complete and can display the need for teacher and student alike to seek new information for the prevention and cure of these disabilities. Truly, he creates a way of life. Francis Blake was this type of man—not because he had learned a pattern but because of a spirit from within.

To us as undergraduates, Dr. Blake constantly brought the impression that we were participating in the march of medicine. At times it seemed he had cultivated what Osler called "The art of detachment" to a high degree, but on teaching rounds there was friendliness and encouragement, a willingness to discuss the perplexities, so that the sessions were anticipated eagerly as group activities of enlightenment that were all too short rather than squirming ordeals of exposed ignorance. He was authority, but did not use its voice; the why's and the how's were the theme.

Advancing as a student through the house and junior staffs was a stimulating experience. Attendings, residents, and internes were essentially one as students striving for clinical achievement and understanding. Here again, Dr. Blake was at home with the house staff; any issue could be debated and evidence called for. He did not exhort to do better; it was unbelievable not to try. Rarely did he storm outwardly; as James Trask said at the time of Doctor Blake's fiftieth birthday, "When you pulled a boner, he didn't say much but he made you feel awfully foolish." He was really an eloquent master of the unspoken word.

But amid all the particulars the dominant theme recurred: "How and why do these conditions come about?" One was led to consider the mechanisms of the disease problem and their meaning to the broad field of control. His own studies with pneumonia and influenza, measles and scarlet fever, all had the broader concept. In reply to questions from the junior men, the answer was frequently, "Why don't you try to find out?" It was not a question of whether one was able to do it (or, as now, whether one should first ask for a grant), but was one willing to try? In all this Drs. Peters, Trask, Marvin, Fox, and others were able abettors. It was this incisive, direct approach to clinical investigation, the ability to see the problem and to engage it or to furnish the stimulus and support to others which one came to recognize as an outstanding characteristic.

It was inevitable that the qualities which made Francis Blake so important within his home environment should be sought for other needs. One place where his abilities as teacher and counsellor became a dominant influence was the Army Epidemiological Board. It rapidly brought together a number of students and investigators, many of whom had scarcely worked outside the clinic or the laboratory, and confronted them with the undetermined job of clarifying and seeking to control the problems of infectious disease which might arise in unpredictable fashion wherever our armed forces

might be. It was to him a natural opportunity because of his interest in promoting direct investigation in which the field and laboratory would of necessity be blended and in advancing the studies as rapidly as effective means of investigation could be devised. It was always evidence rather than mere opinion that was accepted as the principle with which to guide development. His interests were those of the scientific mind, and his work in New Guinea was ample evidence of the active research worker teaching by precept. There were many from widely scattered areas who thus came to know Francis Blake. He established a new school of enthusiastic students among these mature investigators through whom his abilities became recognized as an important national asset. And apart from the reaches of Yale Medical School the fine standards he represented have extended in a widened circle to guide investigators who may not even know his name. He gave us of his spirit: devotion to principle, the quest for understanding, the vision and courage for exploration, the beauty of truth, and the valor of integrity. His passing has left a lonely spot on the horizon, but the image of his stature remains strong and clear in our hearts and minds.

THOMAS FRANCIS, JR.

REMEMBRANCE OF DR. BLAKE

Mrs. Blake, members of Dr. Blake's family, and friends gathered together here in memory of Dr. Blake:

A few hours ago I was sitting at a table in the office of General Simmons at the Harvard School of Public Health in Boston with a group of men who had served in the Medical Department of the Army during World War II, and who were holding one of their many post-war meetings as an advisory editorial board on the history of preventive medicine in the Army from 1941 to 1946. At this meeting, as in all other similar meetings and, indeed, as in our work during the war, we were dealing with the thoughts and actions of Dr. Blake. He had such a far-reaching and strong influence over military preventive medicine that the history of those days will be a part of the history of Dr. Blake. Although his name may not be mentioned throughout, those who know what happened and how it was done will see Dr. Blake's figure moving through all the events.

Dr. Francis has just spoken eloquently about Dr. Blake as a teacher of medical students and as a teacher of all physicians who came in contact with him. Although I was never officially on a clinical service under him, I received much informal tutelage from him at Yale, in Washington, and in New York. To enlarge on what Dr. Francis has said, I could give many additional examples of Dr. Blake's influence upon education, research, and Army affairs. To do this I want to start at a point about twenty years ago

when I began to work with Dr. Blake and formed a relationship which changed my whole life, I truly believe.

In 1932 and 1933, when I was chairman of the Division of Medical Sciences of the National Research Council, I and others had a conviction that no one else we knew would be as good as Dr. Blake in the chairmanship of this Division. He was appointed, and ever thereafter, through the rest of his life, he did great and good things for the National Research Council and its Division of Medical Sciences. In addition, he was put in charge of the fellowship program. Once they had got him, they never let him go.

Shortly after that I came to Yale to positions in the Medical School and as Master of Trumbull College. This was a momentous move for me. I know that unless Francis Blake had sanctioned it, the move would never have taken place. After I came here I began to do what anyone else did if he had the opportunity and that was to get help and advice from Dr. Blake on all sorts of problems. He had a keen capacity for analysis and after listening to complicated descriptions he would sum up very clearly what the speaker was trying to tell him and then would say something directly to the point and of great value. Problems I would take to him became really urgent when I was made Dean of the Medical School in 1935. Among the first of these was the recurrent problem of cutting the budget. I used to talk over the painful questions with Dr. Blake. While he had no responsibility for what was done finally, I do owe him thanks for advice and opinion that helped a great deal to make reductions without upsetting the delicate balances that exist in an intricate structure such as a medical school.

Dr. Blake had a zest for puzzles. To solve these was his relaxation at the friendly parties that Dorothy and he used to give at their home, where you were tested by all sorts of puzzles, from arithmetical tricks to equilibration stunts, and where you were applauded if you could balance an apple on the end of a stick that rested on your forehead or chin. I used to think it was in the same way that Francis went at puzzles of balancing the budget, or revising the curriculum, or planning research.

As President of the Army Epidemiological Board from 1941 until 1946, Dr. Blake did a tremendous work. He and General Simmons established this research and advisory board against innumerable difficulties—small and large. Through Dr. Blake's compelling power, aided by the patriotism of the time, the leading authorities in the country who did not have other official governmental connections went to work for The Surgeon General on the investigation and control of influenza and other epidemic diseases. Under his inexhaustible guidance and good sense, there followed remarkable years of war work which gave results that were as good for civilian medicine as they were for military medicine—that had lasting benefits for the welfare of people in peacetime as at the moment they had for the protection, preservation, and maintenance of the health of troops in war.

Through all the stresses, anxieties, puzzlements, and urgent work of the Army Epidemiological Board and of Preventive Medicine Service during the war, I had the great advantage of being close to Dr. Blake, handling things for him, as one would say in the Army, as the administrator of the Board. There was never any troublesome disagreement with him, although during some periods there were strong differences of opinion. As I think back over the hundreds of days of working with him under these conditions, I cannot recall any name-calling or sharp talk from Francis. Many a time he would begin a discussion of something by saying, "B-J, I am a little bit disturbed. . . ." This was said slowly, deliberately, and ominously. Then he would say what he wanted to say and according to his habit could not be interrupted until he had finished speaking. When any interrupting remark was made he could suspend speech while showing by his expression that he was still on the same subject, and as soon as the interruption faded out under his rising voice he would resume the statement of his view. I used to think this was another characteristic of his intentness, singleness of purpose, and clarity.

Much of what I have said has had to do with organizations, official relationships, research problems, administration, and rather abstract matters. It is not inappropriate because Dr. Blake seemed to me to be an abstract, rather remote, and highly intellectual person. Coming from Louisiana I used to view him with awe and some fear as a New England Manifestation. Certainly I realized that when I praised or complimented people with what I thought was only moderate Southern enthusiasm, he regarded such expressions as bordering on the untruthful, to use a mild term. He reserved his commendation for only the best. When he gave it, the one who was lucky enough to receive it knew it was pure gold, and treasured it as the coin of the realm.

We who worked with him in war and peace will remember him for his extraordinary abilities and qualities. He never seemed to get tired, although we knew how weary he must have been many times. He never personally complained of troubles, yet some of us know how vexed he was by some stupid and unnecessary situations of discomfort. He never shirked responsibility. In the morass of policy determinations he showed the same courageous search for the truth as he did in the brave scientific work he carried on in the jungles of New Guinea in the search for the cause of scrub typhus. He was a dependable friend in all circumstances. However austere he may have been as an official, he was most agreeable as an off-duty companion.

It is not possible to say all that is in our hearts and minds while we are here together for a little while in memory of Francis Blake. Perhaps the intimations are enough. It is good for us to think of him in this room of history and to share sentiments and admirations that will endure.

STANHOPE BAYNE-JONES

FRANCIS GILMAN BLAKE

I take it we are not here to enumerate the triumphs of Francis Blake and the various positions of responsibility and honor to which he was called. These are matters of public record. We are glad that his ability was recognized and proud of, but not surprised at, his extramural achievements. At times, as we saw his sphere of action expand, many of us experienced a little sense of regret, sometimes approaching resentment, that we could enjoy less continuously the intimate companionship and personal counsel on which we had learned to depend. These feelings were rather rueful than selfish and in themselves constitute a tribute to a personal character that we cherish. It is natural and proper that one who came with Francis Blake to Yale, as a member of the faculty of his department, should choose to dwell on these personal characteristics and the influence he had on his fellows and the development of the School of Medicine.

Sometimes the record is cited in terms which imply that at the eventful moment of Francis Blake's appearance in New Haven the School of Medicine had fallen to such low estate that it was all but defunct. This may be intended as flattery to those who raised it from this low estate to its present position of honest and distinguished poverty. It may be only one more of those plausible but specious rumors that emanate at intervals from the red elements on the banks of the Charles, who could not be expected to recognize that Francis Blake's primary allegiance was to Dartmouth's rugged hills. In any case, sows' ears are still unlikely materials. There were in Yale spirits endowed with healthy self-criticism and imagination. And one signal mark of this imagination was the selection of Francis Blake as Professor and Executive Head of the Department of Internal Medicine at the tender age of thirty-four. Although he had achievements beyond his years to his credit, this was an unprecedented appointment in the days when pontifical gray beards were still the academic vogue. Francis Blake was one of those who changed the styles. Without ostentation he demonstrated in a short time that he was not only a competent, but an astute, clinician, a most essential feature in the day when the old school insisted that science was incompatible with the bedside manner. His success established precedents.

His clinics were models of organization, not from the standpoint of stage properties and display, but as examples of the orderly array of the presenting facts, their critical evaluation, and their correlation with other known facts culled from the literature and experience. Also, he was not afraid to foster the spirit of inquiry by speculation; but this was carefully considered and ingenuously labeled.

Part of his effectiveness in these and other public meetings lay in an acuté analytical sense that enabled him to reach with unusual rapidity the

core of a problem or question, coupled with the ability to express himself with incisive clarity and force. The same perquisites he displayed at the bedside, whether teaching, making rounds, or consulting with his associates. In those days when both the student body and the faculty were small, the professor took his tour of routine rounds as well as consulting rounds. There were few distracting outside activities. I hope the informal atmosphere built in those first years by Blake and his associates will survive the continuing expansion of the School because it is, I believe, one of the finest elements in the flavor of Yale.

While he was improving the quality of practice in the hospital, he did not neglect the pursuit of scientific investigation, thereby again proving that science and practice need not be divorced. His stimulating spirit was, moreover, not confined to his own field. Where he saw a spark he fanned it. His own investigative achievements are recorded in his voluminous bibliography. His influence on others is evidenced in the men that were attracted to his department and his medical service in the hospital, and the large proportion of these who have elected to follow careers of education and research. One of his outstanding disciples, Tommy Francis, has spoken to you today.

Francis Blake had a great faith in freedom in all things, perhaps particularly in education. He believed that education was a product of learning and that men achieved it best if they were given opportunity and encouraged to grasp it. In this spirit he abolished lectures in course by a process of attrition and was the leader in transposing the conventional sequence of ward and dispensary services in the clinical curriculum. He stimulated the members of his department to pursue investigation, encouraged their efforts, and provided facilities generously. But he did not try to channel their explorations according to his own predilections. He seldom offered gratuitous advice and was slow to rebuke his fellows, sometimes giving the impression of aloofness or lack of interest; but if his advice was asked, it was given freely. He had a most acute and discriminating critical sense. To me he demonstrated most clearly that the rules of logic as applied to the analysis of evidence are not categorical, not limited by the boundaries of a man's field of experience or expertness; they are universal. Although our lines of exploratory adventure were quite divergent, I early learned to set a high value upon Francis Blake's opinion of my work and to seek his criticism of my papers in advance of publication.

His love of freedom was coupled with a happy measure of tolerance. He held positive views and expressed them in a forthright manner that sometimes seemed uncompromising; but he was seldom opinionated or contentious. Inevitably, he differed sharply at times with almost all of his associates; but these differences seldom created prejudice. I am aware that

Francis Blake was not wholly sympathetic with some of my opinions and the direction of some of my efforts; but, in the thirty years of his administration, I enjoyed not only freedom, but support in the pursuit of my aims. This is mentioned not to stress these differences, because they were, to me, insignificant in comparison with our common interests; but as an expression of appreciation of Francis Blake's tolerance and love of freedom which was one of the solid roots of my deep affection for him.

It is natural that with these attributes Francis Blake's counsel should have been sought on every important issue in the development of the School of Medicine; that he should have held so many posts of responsibility and honor in the School, including the deanship in the turmoil of war. It is equally natural that his talents could not be limited to the narrow confines of this School. Although his value deepens the tragedy of our loss, the imprint he has put upon us at the Yale School of Medicine is ineradicable.

JOHN P. PETERS

FRANCIS GILMAN BLAKE AWARD

The Beta Zeta chapter of Nu Sigma Nu fraternity in the Yale School of Medicine has established a memorial to be called the Francis Gilman Blake Award, honoring the Sterling Professor of Medicine who died on February 1, 1952. This announcement was made at the reception for the graduating class given on May 25, 1952 by Dean C. N. H. Long and Mrs. Long.

The terms of the award state that it will be given annually to the member of the faculty of the Yale University School of Medicine designated by the graduating class as the most outstanding teacher of the medical sciences. The first recipient is Dr. Gerald Klatskin, Associate Professor of Medicine, who was trained by Dr. Blake and, since the war, had been one of his associates in the Department of Internal Medicine.

Dr. Klatskin was born May 14, 1910, in New York City, and received both B.A. and M.D. degrees at Cornell. Except for the period from January 1, 1935 to June 30, 1937, spent at Strong Memorial Hospital, Rochester, New York, Dr. Klatskin received his postdoctorate training under Dr. Blake in the New Haven Hospital as Interne, Assistant Resident, and Resident. He was in private practice in New Haven from 1938 to 1942 and served as Clinical Instructor in Medicine at Yale. Having entered the Medical Reserve Corps in 1935 he was on active service from July 1, 1942 to April 4, 1946. Two years of this duty were spent in India and then as a Lieutenant Colonel he was Chief of the Medical Service at Schick General Hospital, Clinton, Iowa. He is now a Senior Consultant in Medicine to the Veterans Hospital in Newington, Connecticut. He has been an associate professor at Yale since 1948 and, in addition to his effective teaching, evidenced by this award, carries on a varied program of research dealing primarily with liver disease.

EVENTS

BEAUMONT CLUB

April 18, 1952

MALPIGHI AND HIS THEORY OF PRE-EXISTENCE. By Howard Adelmann, Professor of Histology and Embryology, Cornell University.

Marcello Malpighi (1628-1694) had at the age of twenty-five already received his doctorate of medicine and philosophy degrees. At twenty-eight he was the professor of medicine at the University of Bologna.

Among his accomplishments we find that he discovered the alveoli in the lung and observed red blood corpuscles in the blood vessels of the omentum of the hedgehog (at the time he interpreted them as fat droplets). He described the stratum germinativum of the epidermis, and the fibers of the central nervous system which he thought were glandular ducts; he discerned the glandular nature of the liver, described the architecture of the kidney, pointed out the contractile, sponge-like nature of the spleen, and was actually the first to describe the epoöphoron, Gartner's duct, and the anatomy of the metamorphosing silkworm. Malpighi was also the first to remove the entire chick blastoderm and study it on a glass slide and to describe the neural folds in the chick embryo. His observations and descriptions of the embryology of the chick remained unsurpassed for 150 years.

Because he observed an embryo in an egg which he believed to be unincubated, he felt that the embryo "pre-existed." His theory of pre-existence however, bore more similarity to the modern concept of epigenesis than to theories of pre-formation or enboîtement. He conceived of the egg as supplying the amorphous material for the formation of the embryo and the sperm as providing the "plastic spirit." Even with our use of the terms organizer, inductor, and field, one wonders just how far we have progressed beyond Malpighi in our understanding of the forces fashioning and directing the development of the embryo.

Although some of his interpretations were incorrect, the extent and accuracy of his observations, made without the aid of fixation, staining, or injection techniques, must arouse our deepest esteem and admiration for this great scientist.

J. M. Q.

PLANT SCIENCE SEMINAR

April 21, 1952

DEVELOPMENTAL STUDIES OF BIOCHEMICAL CHARACTERS IN PLANT HAIR CELLS. By Milton L. Zucher, Washington University.

The hair cells of *Coleus* plants afford an opportunity to study cellular differentiation on a very simple level. Each of these hairs is derived from a single cell, and consists, when mature, of cells which are all of the same type, differing from one another only quantitatively.

An epidermal cell, by a series of divisions, produces a single file of cells, tapering from base to tip. Only the terminal cell of the growing hair divides. Cell elongation begins terminally and progresses basally. A study was made of the development of pigmentation in the growing hair. The anthocyanins and flavenoids were determined from cell extracts, and compared by means of a microspectrophotometer with the pigments of individual growing cells. It was found that anthocyanin formation follows the wave of cell elongation from tip to base, but is slower, reaching the middle of the hair by the time

elongation is completed. Flavenoid production, on the other hand, proceeds simultaneously in all cells. The pigment concentration in mature hairs is constant from cell to cell; the ratio of amount of pigment to cell volume is essentially the same as that of nuclear volume to cell volume.

Tissue culture experiments showed that it is not a simple gradient of nutrient concentration which is responsible for the decrease in size of each cell cut off in the growth of the hair.

It has been found that X-rays and ultraviolet light cause unusual patterns of pigment development. These are now being studied.

R. R. L. G.

PHARMACOLOGY SEMINAR

April 24, 1952

THE PHYSIOLOGICAL AND CLINICAL IMPORTANCE OF AMINE OXIDASE. By J. H. Burn, Professor and Chairman of the Department of Pharmacology, Oxford University.

Monoamine oxidase has been demonstrated in such sympathetic-innervated structures as the nictitating membrane, pupil, and blood vessels of the cat by manometric measurement of O_2 uptake in homogenates. Tyramine was used as the substrate. It was shown that the enzyme has greater activity on nor-epinephrine than on epinephrine by incubating a fifty-fifty mixture of the two substances with the amine oxidase and biologically assaying for their ratio before complete breakdown had taken place. This fact may explain why nor-epinephrine gives a shorter and lesser response on the nictitating membrane than that obtained with epinephrine, since the latter would be destroyed at a slower rate. It was also shown that after denervation this difference in activity is almost completely abolished, in spite of the fact that the enzyme drops by only 40 per cent by the tenth day, and then slowly rises to normal levels. The possibility of reinnervation has to be considered here.

Thyroid extract was shown to depress liver amine oxidase, permitting more epinephrine to exert its glycogenolytic effect and resulting in a greater hyperglycemic response. Thyroidectomy, on the other hand, produced the opposite results.

On the separated and perfused rabbit-ear a difference in effect has also been demonstrated, 5.9 times as much nor-epinephrine being necessary to give the vasoconstrictor effect produced by epinephrine. After ephedrine this ratio drops to 1.7, presumably because of inhibition of amine oxidase activity besides a direct stimulatory effect. Cocaine, amphetamine, and desoxy-amphetamine are considered purely inhibitory on the enzyme, their facilitatory and stimulating actions being thus due to interference with the breakdown of the nor-epinephrine released at the neuro-effector junction.

N. A. H.

THE FIFTEENTH HARRY BURR FERRIS MEMORIAL LECTURE

April 30, 1952

THE RÔLE OF THYROID STRUCTURES IN THE ELABORATION OF THYROXINE. By Charles P. Leblond.

The hypothetical sequence of events in the elaboration of circulating thyroxine can be conveniently divided into five steps as follows: concentration of iodide in the following cells; transformation of iodide into thyroglobulin; temporary storage of thyroglobulin in the colloid; enzymatic

hydrolysis of thyroglobulin to thyroxine; secretion of thyroxine into the circulation.

No evidence was presented to relate the first step to any morphological structure. Regarding the second step, a new technique of applying photographic emulsion directly to histological sections showed that at one hour after the injection of radioactive iodine into normal rats there was photographic evidence of a concentration of thyroglobulin in the apices of the follicular cells. That these particles were not merely iodide was presumed from the fact that iodide would have been dissolved and lost during the histological staining process. Likewise for the third step, twenty-four-hour-post-injection specimens revealed a similar concentration of thyroglobulin in the colloid of the follicle instead of in the cells. It was further shown that rats on a low-iodide intake or on pituitary extract intake concentrated thyroglobulin in the colloid within an hour, whereas hypophysectomized rats showed thyroglobulin in the follicular cells as late as twenty-four hours.

The validity of the hypothesis that hydrolysis results in the elaboration of many iodinated amino acids in addition to thyroxine was indicated by studying radio-autographic records of two-way filter paper chromatograms of thyroid extracts and plasma. Mono- and di-iodotyrosine, triiodothyronine, and thyroxine as well as several other unidentified iodine-containing compounds were found in the colloid or thyroid tissue; thyroxine alone was found in the plasma.

No evidence was proffered to indicate the exact anatomical site of the fifth step.

J. F. S.

NU SIGMA NU LECTURE

May 1, 1952

THE PATHOLOGIC PHYSIOLOGY OF COR PULMONALE AS RELATED TO DIAGNOSIS AND TREATMENT. By C. K. Friedberg, Chief of Cardiology, Mount Sinai Hospital, New York, New York.

With the development of quantitative methods, cardiorespiratory physiology has become susceptible to more precise study in recent years. In cor pulmonale, the lungs as well as the heart become decompensated. Pulmonary insufficiency may be defined as a condition in which the oxygen saturation of the blood decreases below normal levels. The etiological conditions leading to this insufficiency fall into several groups: (i) emphysema (obstructive), (ii) fibrosis with or without emphysema (restrictive, obstructive), (iii) fibrosis (alveolar, capillary), and (iv) vascular obstruction. Each group may involve a different physiological mechanism but all may cause pulmonary decompensation resulting in low oxygen tension in the arterial blood and cyanosis. Furthermore, in some instances, a concomitant insufficiency of the right heart, attended by its well-known clinical syndrome, may develop. In chronic cor pulmonale, the two common factors affecting the heart are elevated pressure in the pulmonary vascular bed and reduced oxygen saturation of the arterial blood. The observation that the pulmonary vascular resistance rises during exercise suggests that right-sided heart failure may be somewhat analogous to left-sided failure in systemic hypertension. Digitoxin tends to improve quantitatively the output of the right ventricle and to relieve the clinical symptoms referable to heart failure, but it does affect the cyanosis. An explanation, therefore, of the development of cor pulmonale must begin with the poor aeration of the blood. The anoxia of the blood going to the tissue sets up reflexes which

increase cardiac output. The heart is burdened further by pulmonary hypertension in the diseased lungs, and "high-output failure" follows.

Diagnosis rests on the demonstration of primary pathology of the lung. Decompensation occurs under some strain, commonly an infection. Therapy is directed toward both the precipitating factor and the myocardial insufficiency. Cardiac treatment, including digitoxin, helps the heart meet its obligation to supply more blood to the tissues, but recovery of the heart and of the patient is dependent on the reëstablishment of pulmonary compensation. Oxygen therapy is used with care not to produce respiratory depression. Phlebotomy, after the acute phase, may ease the load imposed by polycythemia, but is not in itself a curative measure.

O. L. K.

PHARMACOLOGY SEMINAR

May 8, 1952

POTASSIUM AND SODIUM IONS IN NERVE PHYSIOLOGY. By R. Lorente de Nó, Rockefeller Institute for Medical Research, New York, New York.

The observed effects of potassium on depolarization of the nerve are of pharmacological rather than physiological significance. If potassium were of import in physiologic excitability, the ion should depolarize the nerve instantaneously, whereas it actually requires several hours. Furthermore, hypertonic solutions of either sodium chloride or glucose prevent the potassium depolarization. The nerve fibre, it appears, is maintained more by active metabolism than by substances in the surrounding nutrient medium.

After the nutrient medium is deprived of sodium for several hours the intact nerve loses its excitability; removal of the epineurium, a process which produces constriction and basophily of the axoplasm, reduces the onset of inexcitability to five or six minutes. A number of quaternary ammonium compounds containing two or more ethyl groups (or larger radicals) are able to substitute for sodium. Also able to restore excitability of nerve in a sodium-free medium are quaternary ammonium compounds extracted from brain. It is hypothesized that sodium plays a rôle similar to a coenzyme and aids in synthesizing quaternary ammonium compounds necessary for normal nerve activity. These substances are lost in a sodium-free medium.

J. P. G.

NEUROLOGICAL STUDY UNIT

May 19, 1952

BODY IMAGE IN NEUROLOGY. By McDonald Critchley, Dean of the Institute of Neurology, National Hospital, London.

The conceptual scheme of one's body image is frequently a denial of objective fact. Body image development lags behind anatomical and physiological development. Thus elderly people, although intellectually aware of their age, picture themselves in their youth, as demonstrated in the extreme by the fervor with which faded beauties attempt to cling to their former glamor. Moreover, giants and midgets construe their heights as normal, bald men picture themselves well covered, and persons afflicted with scoliosis imagine themselves erect.

The most dramatic demonstration of distorted body image is seen in the "phantom limb" syndrome. The image of a missing part of the body persists not only for limbs, but also for the nose, rectum, penis, etc. However, there seems to be no persistent image of the breast after mastectomy.

Certain other pathological and psychological states also distort the body image. Hypochondria and localized pain increase the size of the image of the afflicted part; electric shock decreases the total body image; various ecstatic states, induced by Hashish intoxication or even music, distort the body image. The image of an orchestra conductor assumed enormous proportions during moving musical passages, until he envisioned himself conducting while astride the world. On the other hand, parietal hemiplegia may result in the loss of the image of the affected side of the body to an extent that may lead to its denial or neglect. Depersonalization may engender visions of imaginary people as well as denial of real images.

The body image may project beyond the body to include inanimate objects like clothes, or the iron lung in the case of a victim of bulbar poliomyelitis. An automobile driver extends his image to all four fenders, a surgeon to the tips of his instruments.

So profuse are the different forms of the body image, even in normal people, that one wonders which image is raised in resurrection.

G. McK.

SEVENTH ANNUAL ALPHA KAPPA KAPPA LECTURE

May 19, 1952

HEADACHE MECHANISMS. By Harold Wolf, Professor of Medicine and Associate Professor of Psychiatry, New York-Cornell Medical School.

The most common complaint presented to physicians, the headache, results from a variety of mechanisms such as displacement or distension of pain-sensitive intracranial structures, distension and edema of branches of the carotid artery, prolonged contraction of muscles of the scalp and neck, painful stimulation of the mucous membranes, and pressure from intracranial tumors.

Six basic mechanisms contribute to headaches that originate from intracranial sources: (i) traction on the sinuses and its tributary veins, (ii) traction on the middle meningeal artery, (iii) traction on the Circle of Willis, (iv) inflammation of pain-sensitive areas of the dura, (v) dilatation of pain-sensitive arteries, and (vi) direct pressure on the fifth, ninth, and tenth cranial nerves. Traction and displacement of these pain-sensitive structures may accompany increased intracranial pressure, or the decreased intracranial pressure often resulting from spinal taps. Moreover, the generalized throbbing headache seen in anoxia, "hangover," and fever, and experimentally produced by the intravenous injection of histamine, follows increased pulsation and distension of the intracranial arteries. Headaches that result from these mechanisms can be "spun out" by a "human centrifuge" that decreases cranial pressure and blood flow.

Migraine headaches, associated with distension of extracranial arteries, appear to have three phases: (i) vaso-constriction of the arteries producing blanching and occasionally visual defects—a pre-headache phenomenon, (ii) painful vasodilatation, and (iii) edema of the vessel walls. Small vascular accidents often accompany the second and third phases.

Psychological factors play a significant rôle in almost 80 per cent of all headaches. Many migraine attacks can be relieved by removing the psychological disturbances, headaches associated with hypertension are often alleviated without modifying the hypertension, and hay fever attacks can be induced and relieved by psychological suggestion.

G. McK.

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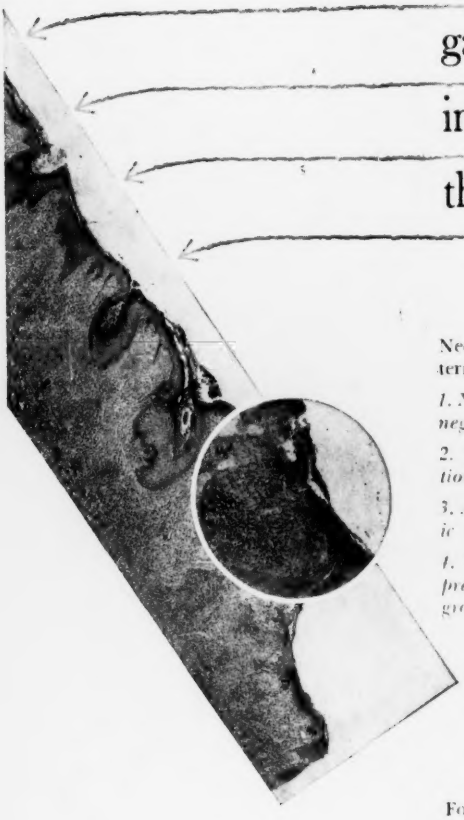

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